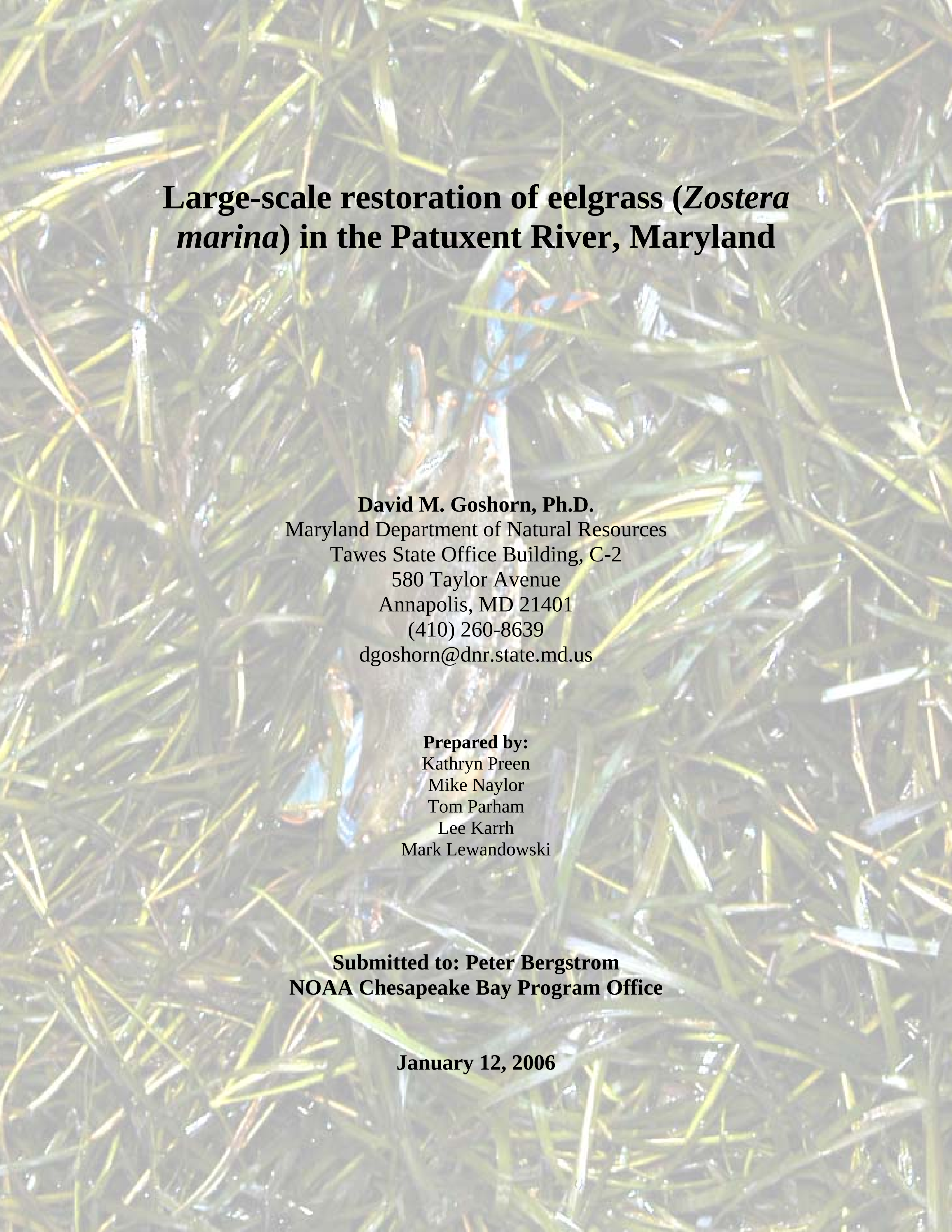

Large-scale restoration of eelgrass (*Zostera marina*) in the Patuxent River, Maryland

David M. Goshorn, Ph.D.
Maryland Department of Natural Resources
Tawes State Office Building, C-2
580 Taylor Avenue
Annapolis, MD 21401
(410) 260-8639
dgoshorn@dnr.state.md.us

Prepared by:
Kathryn Preen
Mike Naylor
Tom Parham
Lee Karrh
Mark Lewandowski

Submitted to: Peter Bergstrom
NOAA Chesapeake Bay Program Office

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Recipient: Peter Bergstrom, Ph.D.
Fishery Biologist, NOAA Chesapeake Bay Office
410 Severn Avenue Suite 107A
Annapolis, MD 21403

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St. Mary's College

PI Contact information:

David M. Goshorn, Ph.D.
Maryland Department of Natural Resources
580 Taylor Avenue, C-2
Annapolis, MD 21401
Phone: (410) 260-8639
E-mail: dgoshorn@dnr.state.md.us

Project Team: Kathryn Preen, Natural Resources Biologist, Maryland DNR
Tom Parham, Chief Living Resource Assessment, Maryland DNR
Mike Naylor, Senior Biologist, Maryland DNR
Lee Karrh, Senior Biologist, Maryland DNR
Mark Lewandowski, Natural Resources Biologist, Maryland DNR
Rebecca Raves, Natural Resources Biologist, Maryland DNR

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Executive Summary (Patuxent SAV planting)

This report details the methods, results, and conclusions from the MD-DNR 2004-2005 large scale eelgrass restoration efforts on the Patuxent River. Because of the essential functions that SAV serve in maintaining a healthy Chesapeake Bay ecosystem: producing oxygen, providing food for a variety of animals and waterfowl, providing shelter and nursery habitat for juvenile fish and crabs, and reducing pollution and improving water quality by absorbing nutrients and trapping sediments, the U.S. Environmental Protection Agency's (EPA) Chesapeake Bay Program (CBP) adopted a goal seeking to increase SAV acreage in the Chesapeake Bay to 185,000 acres by 2010, with an additional goal to plant 1,000 acres by 2008. In order to meet the latter goal, large scale SAV restoration efforts were initiated. This project was initiated to conduct large scale restoration of eelgrass in the Patuxent River, MD by dispersing seeds using two methods: spring seed bags and fall seed broadcasts.

- For the spring seed bag method, eelgrass reproductive material harvested from healthy donor beds in the lower bay was deployed within a few days using a buoy-deployed seeding system (BuDSS) allowing seeds to fall out naturally in suitable shallow water habitat.
- For the fall seed broadcasts, reproductive shoots were held throughout the summer to allow seeds to separate from reproductive material as they matured for later broadcast.

At five restoration locations in the Patuxent River, 23.75 acres have been seeded using both methods since the start of this project in 2003. In addition to seed dispersal, adult eelgrass shoots were planted in test plots to monitor the survival of adult plants at the seeding areas.

Some technical problems were encountered with the seed processing and storage procedures, and the germination and survival rates of the planted seeds were both low. High mortality of seeds held over the summer limited the acres that could be planted using fall seed broadcasts. Although no seedlings germinated (recruited) from the fall seed broadcast method, 874 total seedlings were recruited using the spring seed bag method. Increases in turbidity and temperature during the 2005 eelgrass growing season appear to be responsible for the demise of all of the recruited plants (seedlings) and adult test plot plants in the late summer.

Despite the problems in seed processing and storage, and the loss of all recruited seedlings that occurred, our understanding of the physical and chemical processes that affect eelgrass growth and survival in this river has increased significantly. Since seeds have so little stored energy, planting SAV using seeds may only be successful in years that have near-optimal conditions for germination and establishment of seedlings. Since we are currently unable to predict which years these will be, we may need to plant eelgrass seeds for several years at the same sites to have one successful year. Once we have a few successful years with planting seeds, we will be better able to predict when these years are likely to occur, and increase our SAV planting from seed efforts in those years.

Introduction

Submerged Aquatic Vegetation (SAV) is a group of rooted, vascular macroscopic aquatic plants found throughout the shallow tidal and non-tidal waters of the Chesapeake Bay and its tributaries. SAV serves many essential functions in maintaining a healthy Chesapeake Bay ecosystem including: producing oxygen, providing food for a variety of animals and waterfowl, providing shelter and nursery habitat for juvenile fish and crabs, and reducing pollution and improving water quality by absorbing nutrients and trapping sediments. Studies and historical documents indicate that until the early 1970's, SAV had been continuously present in many regions of the Chesapeake Bay, and the Patuxent River specifically, for the past 1200 years. The Patuxent River estuary, like many other temperate estuaries, exhibited dramatic declines in the abundance of SAV during the later half of the 20th century coincident with increasing population density and nutrient loading within the watershed (Den Hartog and Polderman 1975; Orth and Moore 1983; Cambridge and McComb 1984; Orth et al. 1994). A study utilizing pollen dated sediment cores found that at three locations on the Patuxent estuary, SAV was found continuously from approximately 1200 AD to the early 1970's- at which time seeds disappeared from the sediment record (Brush and Hilgartner 2000).

In the late 1960s and 1970s, increases in nutrient and sediment inputs from development of the surrounding watershed (Kemp et al. 1983) contributed to a sharp decline in SAV populations baywide (Orth and Moore 1983). SAV populations began to rebound in

1984 increasing from 38,000 to 90,000 acres in 2002. However, a dramatic baywide decrease was seen in 2003 when SAV populations declined to less than 65,000 acres.

The Chesapeake Bay Program and SAV

Because of its role in providing habitat, retaining sediment and improving water quality, the restoration of SAV has become an important component of the U.S. Environmental Protection Agency's (EPA) Chesapeake Bay Program (CBP) goals. Over the past 20 years, the CBP has committed significant resources to determining the causes for SAV decline and to identify the best course of action for protecting and restoring natural populations.

In 2003, the CBP adopted a goal seeking to increase SAV acreage in the Chesapeake Bay to 185,000 acres by 2010. The CBP simultaneously created the "*Strategy to Accelerate the Protection and Restoration of Submerged Aquatic Vegetation in the Chesapeake Bay*". The Strategy, the result of more than a yearlong effort among Chesapeake Bay SAV researchers and managers, identified the major actions necessary to successfully increase SAV populations in the Bay. These actions fall into four major categories:

1. Improve water clarity sufficient for supporting healthy SAV populations
2. Protect existing beds from impacts by anthropogenic sources and exotic species
3. Plant or reseed 1,000 acres in strategic locations by December of 2008
4. Conduct applied research and public education / outreach on the benefits of healthy SAV beds.

Large scale restoration efforts were deemed necessary to accomplish Action #3 outlined in the Strategy: to plant or reseed 1,000 acres in strategic locations by December of 2008, and ultimately to meet the CBP goal of 185,000 acres, baywide, by the year 2010.

Patuxent River

As of fall 2005, the Patuxent River is one of only a few sites in Maryland and Virginia that has undergone the two-year site selection process (test plantings and water quality monitoring) outlined as a requirement of the strategy for large scale restoration locations. The Patuxent is the largest river completely in the State of Maryland, draining 932 square miles of land from portions of St. Mary's, Calvert, Charles, Anne Arundel, Prince George's, Howard, and Montgomery Counties (Patuxent River Commission Staff 2003). It is one of the most intensively monitored and modeled rivers of its size in the world, and therefore, serves as an important proving ground for many of the CBP initiatives (Maryland Department of Natural Resources 2005).

Prior to the decline of SAV beds in Chesapeake Bay between the 1960's and 1970's, the Patuxent River supported diverse populations of SAV including *Zannichellia palistris*, *Ruppia maritima*, *Potamogeton perfoliatus* and *Zostera marina* (Brush and Davis 1984). Both stratigraphic records and groundtruthing evidence suggests the presence of *Z. marina*, eelgrass, historically throughout the mesohaline portion of the Patuxent River. The disappearance of these species coincided with the degradation of water quality across the Bay region during this period. Like all other tributaries of the Chesapeake Bay, the Patuxent River has been impacted by nutrient pollution. Excess nitrogen and phosphorus stimulate algae growth. The combination of excess algal growth and suspended sediments can increase light attenuation in the water column and inhibit the growth of SAV. Managers have set forth nutrient reduction goals and have addressed sediment pollution to promote the resurgence of submerged aquatic vegetation and improve habitats (Patuxent River Commission Staff 2003). Nitrogen loads in the Patuxent River were reduced from 5.02 to 4.07 million pounds a year (19%) and phosphorus was reduced from 0.51 to 0.27 million pounds a year (47%) from 1985-2003 (Patuxent River Commission Staff 2003).

A resurgence of SAV in the tidal freshwater reach and middle portions of the Patuxent River since 1993 has been attributed to significant reductions in pollutant loads and resulting improvements in water clarity (Naylor and Kazyak 1995). The 2004 aerial survey recorded 220 acres of SAV in the tidal fresh portion, 4,340 percent of the goal for this portion of the river, and 106 acres in the middle portion, or 156 percent of the goal for that area (Maryland Department of Natural Resources 2005). However, SAV populations remain sparse in the lower mesohaline region of the Patuxent. Only 142 acres were mapped in 2004, far below the 1,325-acre goal for this portion of the river (Maryland Department of Natural Resources 2005) (Figure 1).

Recent analysis by Stankelis et al. (2003) has suggested that the mesohaline portion of the Patuxent River may be inappropriate for SAV restoration based on the reduction of light available to SAV. In the mid-mesohaline portion (just below Broomes Island), continued poor water quality (low secchi depth measurements) was suspected to be responsible for losses and lack of revegetation of SAV. In the lower mesohaline region (near Solomons Island), secchi depth measurements indicated water quality conditions suitable to sustain SAV growth. However, significant light attenuation due to high epiphyte loading was thought to cause the loss of SAV from this area.

Epiphyte test strips deployed in the lower mesohaline region (Drum Point to Solomons Island) have shown elevated light attenuation rates, between 30-70 % of surface light, high enough to cause a loss of plants due to leaf-surface light attenuation (Stankelis 2003). Despite this, habitat assessments and initial test plantings (small eelgrass plots,1-3 m^2) over the past three years have provided evidence that water quality in this same region (Broomes Island to Drum Point) of the river could support eelgrass beds (Maryland Department of Natural Resources and Dr. Walter Boynton, University of Maryland, unpublished data). Water quality data from 1985-2003 analyzed by the Chesapeake Bay Program Tidal Monitoring and Analysis Workgroup indicated that in the lower Patuxent River, total suspended solids, nitrogen levels, and percent light at leaf (PLL, the amount of ambient surface light required at the leaf surface to support growth) all pass the SAV habitat requirements, while light attenuation and chlorophyll levels remained below satisfactory levels (Maryland Department of Natural Resources 2005) (Figure 2). In addition to epiphyte loading, predation, specifically damage by mute swans (*Cygnus olor*) and cownose rays (*Rhinoptera bonasus*), may hinder the success of SAV (Orth 1975). Furthermore, the nature of the test plots- small, low-density plantings, has made them particularly susceptible to both physical damage and epiphyte loading.

The combination of documented historical eelgrass coverage, water quality meeting the SAV habitat requirements according to the SAV targeting system (Parham and Karrh 1998), and the vast water quality dataset for the Patuxent River make this river a prime candidate for large scale eelgrass restoration.

Eelgrass in Restoration

Eelgrass was widely distributed in parts of the Patuxent River and Chesapeake Bay until the late 1960's (Brush and Davis 1984; Orth et al. 2003). Eelgrass was also identified in the SAV Strategy as one of two species with great potential for large-scale restoration in the Chesapeake Bay. This perennial seagrass is capable of both vegetative and sexual reproduction. Reproductive structures form when water temperatures reach 10-15^{o}C and seed production begins when average temperatures reach 15-20^{o}C (Granger et al. 2002), typically late May through early June in the Chesapeake Bay (Silberhorn et al. 1983). Seeds can be released from reproductive shoots in close proximity to the parent bed or shoots with mature seeds still intact may also break free from the plant and be exported from the bed (Orth et al. 1994), serving as a vehicle for long distance dispersal (McCroy 1968). Upon release from reproductive shoots, mature, negatively buoyant seeds fall to the bottom of the water column or are transported from the bed (Orth et al. 1994). Eelgrass seed germination in the Chesapeake Bay appears to be dependent upon water temperature, burial of seeds, and oxygen concentrations (Orth and Moore 1983; Moore et al. 1993), and typically begins in mid-October when water temperatures drop below 15 ^{o}C (Moore et al. 1993).

Early restoration efforts involved transplanting adult eelgrass plants from healthy source beds to restoration locations. Averaging 37,000 dollars per acre (Fonseca et al. 1998) plus additional cost for monitoring, this transplanting method is expensive and labor intensive, and often resulted in minimal success. While transplanting is still utilized as a restoration method, there has been increasing interest in using eelgrass seeds for restoration. Seed broadcasting appears to be a more efficient and cost effective restoration technique (Orth et al. 2000) with the added benefit of having less impact on donor beds.

To collect seeds for use in restoration efforts, reproductive shoots are harvested from healthy donor beds when mature (Granger et al. 2002). Seeds are held in large tanks under ambient conditions until mature, separated from reproductive spathes, and stored for up to 3 months (Granger et al. 2002). Seed dispersal (Orth et al. 1994) typically takes place from mid-August to mid-October before water temperatures drop below 15°C (Orth and Moore 1983; Moore et al. 1993).

Recently, an alternative to traditional seed collection and storage has been developed. Using techniques similar to the buoy-deployed seeding system (BuDSS) developed by Pickerell et al. (2003; 2005), freshly harvested reproductive shoots are placed in mesh bags immediately after harvest, moved to the restoration location, attached to anchored buoys, and deployed in the area to be restored. The mesh bags remain suspended at the top of the water column, allowing the seeds to develop, mature, and drop out over a period of weeks. This mimics the floating and rafting of reproductive shoots during natural seeding events (Pickerel et al. 2003; 2005). This method eliminates the need to store seeds, reducing the number of seeds lost to processing, and decreases the expense and labor requirements associated with seed transport, processing, and storage. Initial restoration efforts using the BuDSS in the Peconic Estuary, NY yielded up to 4% recruitment (Pickerell et al. 2003; 2005).

Transplanted or seeded SAV beds have the potential to thrive and provide benefits similar to naturally occurring beds (Fonseca et al. 1994). There are several regions within Chesapeake Bay in which habitat conditions have shown significant improvement since long term monitoring began in 1985 and are now suitable for SAV recolonization (Maryland Department of Natural Resources). However, many of these regions remain unvegetated due to a lack of SAV seed or propagule sources. By identifying these areas and strategically seeding them, it is hoped that significant numbers of plants will germinate and grow to establish dense, self-protecting beds. The combination of self-protection and reproduction within these beds should generate seeds that may accelerate natural revegetation of areas adjacent to the restored beds (Orth et al. 2003).

The MD-DNR has developed this project to conduct large scale eelgrass restoration at select locations on the Patuxent River, MD. This project will consider previous restoration efforts while investigating new technologies in order to meet project goals and maximize the area that is restored. The following objectives will be met by the conclusion of this project:

1. Identify sites for restoration based on application of GIS based targeting models, recent and on-going test plantings, and intensive habitat assessments.
2. Conduct large-scale seeding of eelgrass at each site over a three-year period as called for in the Strategy.
3. Evaluate associated factors that may influence success of the project such as seeding density, water quality, epiphytic growth, and predation.
4. Produce a final, technical analysis documenting degree of revegetation of each site and evaluating the role of associated factors.

To address these objectives, this project compared the seedling success, as well as the budget requirements, of two seed dispersal methods while simultaneously investigating associated factors that may be contributing to low germination rates and seedling success in the Patuxent River. The first two years of this project were devoted to site selection, which involved applying existing habitat information to identify general areas suitable for restoration and test plantings at specific sites. Large scale broadcast seeding and seed bag deployments were utilized and their relative successes and associated costs were compared. This report presents results and conclusions from the site selection process, the first 2 years of seeding (2003 and 2004), and the relative effects of associated factors on the success of restoration efforts.

Research Methods

Site selection

Locations for large-scale restoration activity were determined using a geographical information system (GIS) based SAV Restoration Targeting System (Parham and Karrh 1998). The model uses six layers of data to evaluate the potential of a particular habitat to support SAV populations. The data layers incorporated into the targeting model included:

1. *Shoreline*: The Maryland shoreline datalayer used was digitized by the Soil Conservation District using United States Geological Survey (USGS) quad sheets at a scale of 1 inch = 24,000 feet.
2. *Water Quality*: The water quality parameter allows site evaluation based on three methods: percent light at leaf, percent light at water (Kemp et al. 1995), or the individual water quality parameters (Dennison et al. 1993). Six water quality parameters important to SAV communities were incorporated into the SAV Restoration Targeting System: light extinction coefficient (Kd), dissolved inorganic nitrogen (DIN), dissolved inorganic phosphorous (DIP), total suspended solids (TSS), chlorophyll a (CHLA), and salinity. Data from a running three year growing season (April to October) for SAV were used to obtain a median value by station for each parameter. The data were obtained from the Chesapeake Bay Mainstem and Tributary Water Quality Monitoring Program as well as several additional spatially targeted water quality programs. The individual water quality parameters were interpolated using the Inverse Distance Weighted interpolation

method in ArcView Spatial Analyst (ESRI) using the four nearest neighbors and 100 foot interpolated cells extending beyond the extent of the Chesapeake Bay. After interpolation of the individual parameters, each parameter was overlayed with salinity coverage and assigned as pass or fail based on the SAV habitat requirements for one meter restoration (Batuik et al. 1992).

3. *Bathymetry*: One and two meter bathymetry contours for the Chesapeake Bay were obtained from the EPA's CBP, intersected with the Soil Conservation District shoreline and converted from lines to polygons. The resulting shapes were designated to yield areas less than one meter depth, areas one to two meters depth, and areas greater than two meters depth at mean low water.
4. *Submerged Aquatic Vegetation*: SAV distribution coverage data was determined based on aerial surveys completed by the Virginia Institute of Marine Science (1981-2004). A composite layer of historical SAV distribution was created by combining the 1981, 1984-1990, and 1991-2004 SAV aerial surveys. Current distribution was based on the 2003-2004 SAV distribution.
5. *Hydraulic Clam Dredging*: Prohibited clamming areas were mapped based on the laws in the Code of Maryland regulating this activity (§4-1037 and §4-1038). MD-DNR natural oyster bar habitats were buffered by 150 feet as called for in the State and County laws. A shoreline setback was established and buffered to the appropriate distance (distance varying by County) using the Soil Conservation District Shoreline coverage.
6. *Blue Crab habitat areas*: These areas were included to encourage placement of SAV restoration activities in areas that are not only suitable for restoration

activities but also provide benefits for species having recognized links to SAV. These areas were defined as outlined in the 1997 Blue Crab Fisheries Management Plan.

A point system is assigned to each data layer and an algorithm developed to evaluate the restoration potential of sites within each tributary based upon the combined datalayers. Figure 3 illustrates a SAV restoration potential map of the Patuxent River based on the SAV Restoration Targeting System.

Study Area

Five sites in the lower Patuxent River; Parrans Hollow (38° 24.714' N 76° 31.649' W), Jefferson Patterson Park (38° 24.438' N 76° 31.268' W), Myrtle Point (38° 19.755' N 76° 29.493' W), Hungerford Creek (38° 20.975' N 76° 28.317' W), and Solomons Island (38° 18.898' N 76° 27.252' W) were identified as suitable habitats for eelgrass recolonization based on the MD-DNR SAV Restoration Targeting System (Figure 4).

Seed Collection

In order to determine the progress of eelgrass seed development and maturation, surveys of reproductive shoot and seed development within potential donor beds in Tangier Sound began in March and continued through April and May of 2004 and 2005. Surveys were conducted by removing a small number of reproductive spadices from plants in possible donor beds and determining the percentage of mature seeds per spadix. Harvesting did not begin until at least 50% of the seeds within reproductive shoot spadices were mature to ensure harvesting takes place during the peak of seed production. As seed maturation progressed through the spring, self-contained underwater breathing apparatus (SCUBA) was used to conduct surveys to directly compare the density of seeds between potential donor beds. Reproductive shoots were removed from plants in a 1 m^2 sample within a potential donor bed. The total number of seeds per m^2 for a given donor bed was determined as the sum of mature seeds per reproductive spadix, the number of spadices per reproductive shoot, and the number of reproductive shoots in the 1 m^2 samples (Inglis and Waycott 2001).

In 2003, reproductive shoots containing mature seeds were collected manually while snorkeling or using SCUBA over eelgrass beds in Tangier Sound (38° 00.530’ N 75° 58.349’ W) (Figure 5). During subsequent harvests (2004 and 2005), a mechanical harvest boat (Pristine Marine, M J McCook & Associates, La Plata, MD) was utilized to increase the efficiency and amount of reproductive material collected. In 2004, seeds were collected from donor beds in the Little Annemessex River (37° 58.479’ N 75° 52.255’ W) and in 2005 from the Little Annemessex River and the mouth of Acre Creek (Big Annemessex River) (37° 59.626’ N 75° 51.636’ W and 38° 01.718’ N 75° 50.632’ W, respectively). Immediately following collection, reproductive material was manually loaded into nylon mesh laundry bags, secured at a nearshore dock, and kept submerged in ambient water overnight. Bags of harvested seed material were transported via commercial waterman vessels to the MD-DNR Piney Point Aquaculture facility in St. Mary’s County, MD within 24 hours of collection. A portion of the harvested material was transferred to seed bags for immediate deployment (2004), while the seed processing procedure began on the remaining reproductive material in order to extract mature seeds for fall broadcast (2003, 2004, 2005).

Test Plantings

In 2003, during the site selection process and again in 2004, alongside seed dispersals, test plantings were carried out to ensure that areas identified by the site selection model (Parham and Karrh 1998) would support growth of eelgrass. Adult eelgrass plants were acquired from Tangier Sound in 2003, Tangier Sound and Chincoteague Bay in 2004, and Chincoteague Bay in 2005. A small portion of the total plants used in 2004 were raised from seed at the Piney Point Aquaculture Facility. Plants were transplanted into three 1 m^2 test plots located adjacent to seed broadcast and seed bag areas. A density of 64 adult plants/m^2 was used for each test plot. Bamboo skewers were used to anchor plants in transplant areas.

Seed Bag Deployment

In 2004, a portion of collected eelgrass reproductive seed material was prepared for immediate deployment following a buoy-deployed seeding system (BuDSS) developed by Pickerell et al. with modifications (2003; 2005). A known volume of reproductive material was subsampled and the number of seeds enumerated. Based on the seed counts, a volume of reproductive seed material necessary to achieve the desired seeding density was transferred to pre-measured, coarse (7 mm) mesh bags, buoys added, and bags fastened securely with cable ties (Figure 6). Two sizes of seed bags were constructed: single (5000 seeds or 100,000 seeds/acre) and double (10,000 seeds or 200,000 seeds/acre). Completed seed bags were transported to planting locations at Parrans Hollow, Myrtle Point, and Solomons Island. On location, a predetermined number of seed bags were attached to cinderblock anchors, and deployed in a grid pattern ranging from 6x5 to 11x11 with 10 meters of spacing between bags. Planting density at each location was estimated to be 37 seeds/m^2 despite varied plot size at each location. Details of 2004 seed bag deployments including location, seeding density, areas covered, are presented in Table 1.

Seed Processing, Storage, and Broadcast

Seed Processing

Upon arrival at Piney Point Aquaculture facility, harvested seed material was emptied from mesh laundry bags into one of eight, 20,000 gallon (32'x32'x4') or one of sixteen 9,800 gallon (20'x20'x4') greenhouse basins. The water in each basin was replaced daily with water from nearby St. Georges Creek and augmented with aquaculture grade sea salt to match conditions at the harvesting areas (~14ppt). In addition, each basin was aerated to prevent anoxia and water quality was monitored twice daily. Typical basin dissolved oxygen levels averaged 5-6 mg/l. Basin water quality data is presented in Appendix A. While in the basins, the eelgrass seeds slowly dropped from the reproductive shoots over the following month. After all the seeds had been released and settled to the bottom of the basins, the seed/reproductive shoot slurry was pumped into a series of stacked settling trays to allow the passive accumulation of seeds while discarding the non-seed material.

Seed Storage

Once separated from reproductive material, the seeds were held in a 2500 gallon tank where water was replaced daily, aerated, and augmented with aquaculture grade sea salt until dispersal in October (2004) or August and October (2005). Seed storage water quality data is presented in Appendix B.

Seed Enumeration

Two methods were utilized to enumerate eelgrass seeds. Estimates of the number of seeds collected and utilized in the construction of spring seed bags were made shortly after collection by counting seeds in four 1L replicate subsamples of reproductive material and multiplying the resulting seeds/L by the total volume of harvested material.

In order to count and determine the viability of seeds to be used for fall seed broadcast, water from the storage tanks was drained completely and the total volume of seed material was measured. The total number of seeds were counted from replicate 2 ml samples of seed material. As seeds were being counted, viability was determined using a "squeeze test" (Orth personal communication 2004). This resulted in a total number of seeds and the number of viable seeds (as a percentage of the total counted) per 2 ml sample. The total number of viable seeds was then extrapolated from this based on the total volume of seeds collected. This procedure was repeated just before broadcast to detect any seed loss that may have occurred during storage and for an accurate measurement of viable seeds for calculating broadcast volumes.

Seed Broadcast

Eelgrass seeds were hand (manually) broadcast (Orth et al. 1994) during the fall of 2003 at the Jefferson Patterson Park location (Figure 7A). A density of 100,000 seeds/acre was used across a total of six 0.5 acre circles (three total acres = 300,000 total seeds). To ensure uniform seed distribution, each of the six circles were divided into 5 m rings. Each ring represented a percent of the total area of the circle. That proportion was used to determine the proportion of seed (50,000 per ring) to be broadcast in that given area (Figure 8). A metal pipe was anchored in the center of the circle and a rope marked at 5 m intervals was attached. Two biologists broadcast proportioned seed aliquots in tandem around one 5 m ring at a time until each ring had been broadcast. Table 2 provides details of the manual seed broadcast at Jefferson Patterson Park in 2003.

A mechanical seed sprayer, mounted to a boat, capable of evenly dispersing seeds at suitable densities (100,000 to 300,000 seeds/acre) at the rate of 10 minutes/acre (C& K Lord, Inc) (Figure 7b), was utilized to broadcast seeds in the Fall of 2004 at the Hungerford Creek, Parrans Hollow, and Solomons Island locations and in 2005 at Jefferson Patterson Park, Hungerford Creek, and Myrtle Point locations. The area of bottom to be planted was multiplied by the desired planting density to determine the total number of seeds necessary. The volume of seeds needed to achieve the desired seeding density was determined based on the percent of viable seeds of the total volume (Orth, personal communication 2004). The flow of the seed sprayer mechanism was then calibrated and adjusted to distribute seeds uniformly at the desired density. Seeds were loaded into the seed broadcast machine and expelled into the water column. All seed broadcasts took place in October before the ambient water temperatures dropped below

15°C, when eelgrass seed germination begins (mid-November to December) (Orth and Moore 1983; Moore et al. 1993). Table 3 provides details of the 2004 and 2005 mechanical seed broadcasts in the Patuxent River.

Surveying and Monitoring

Site Surveys

The survivability of the transplanted, adult, eelgrass plants within test plot areas was evaluated by determining presence or absence of adult plants within the three 1 m^2 test plots at seven months, nine months, and thirteen months after initial planting (May, July, and November). Assessment sof seedling abundance in fall seed broadcast and spring seed bag areas were made beginning in May 2005, seven months after seeding, and the beginning of the eelgrass spring growing season. The eelgrass seedlings were enumerated along two or three non-destructive, 1 m^2 belt transects (Burdick and Kendrick 2001). Using SCUBA, divers completed transects diagonally across the study plots from an offshore corner to the opposite inshore corner using compass bearings. The total number of seedlings along the 1 m^2 transects was then used to extrapolate the number of seedlings present throughout the area (m^2) of the entire seeded plot. Initial planting success was then determined by comparing the total number of seedlings observed to the total number of seeds dispersed in the plot. This same method was repeated in July and November (nine and thirteen months after seeding, respectively) to determine the persistence of seedlings.

The initial planting success was calculated as the proportion of the total number of seeds dispersed that became established (# of seedlings in the sediment confirmed by divers/total number of seeds dispersed) during the first survey (May 2005). The survival of plants was calculated as the proportion of initially established seedlings or adult plants that persisted after nine (July 2005) and thirteen months (November 2005).

Water Quality Monitoring

Spatially intensive water quality monitoring (water quality mapping) was conducted monthly throughout the eelgrass growing season (March - November) throughout the lower portion of the Patuxent River utilizing MD-DNR DATAFLOW systems. DATAFLOW is a shipboard system of geospatial equipment and water quality probes that measure water quality parameters from a flow-through stream of water collected near the water's surface (Madden and Day 1992). Five water quality parameters (water temperature, salinity, dissolved oxygen, turbidity in nephelometric turbidity units (NTU), and fluorescence) were measured. Each water quality datum is associated with a date, time, water depth, and GPS coordinate (NAD83) reported to six decimal places.

Two continuous monitoring (YSI 6600 EDS) stations were located on the Patuxent River prior to and during restoration (2003-2005) to provide temporally intensive habitat assessments to complement the monthly water quality mapping. The first monitoring station was located at the Pin Oak Farm (38° 24.528' N 76° 31.308' W), near the Parrans Hollow and Jefferson Patterson Park restoration sites and the second monitoring station at the Chesapeake Biological Laboratory dock (38° 19.002' N 76° 27.156' W), near the

Solomons Island restoration site. Each continuous monitor recorded seven water quality parameters (water temperature, salinity, dissolved oxygen, turbidity, fluorescence, pH, and reduction potential) every 15 minutes. Both meters were located at a constant depth of approximately one meter below the surface of the water. The continuous monitors were deployed throughout the SAV growing season and data was downloaded weekly during deployment.

Fixed station water quality monitoring cruises were conducted monthly at eleven stations throughout the mainstem of the Patuxent River beginning in 1985 and continuing through 2005.

Detailed information for the Maryland Department of Natural Resources Chesapeake Bay Shallow Water Quality Monitoring Program, including specific methods of the DATAFLOW, Continuous Monitoring, and Mainstem Cruise Programs, can be accessed:
2004: http://mddnr.chesapeakebay.net/eyesonthebay/swm_qapp_2004.pdf
2005: http://mddnr.chesapeakebay.net/eyesonthebay/swm_qapp_2005.pdf

Cost Per Acre and Survival Calculations

At the conclusion of the first year of restoration, several calculations were made. To determine the financial investment made per seed dispersed, the total cost of the particular method was divided by the total number of viable seeds dispersed using that method.

Cost per seed = Total cost associated with method/Total number of viable seeds dispersed

The total cost for restoring one acre could then be calculated by multiplying the cost per seed by the specified seeding density (200,000 seeds/acre). The recruitment success of each method was determined by dividing the total number of seeds dispersed by the number of successfully recruited plants. The total cost for each method was divided by the total number of successfully recruited seedlings to determine a ratio of cost per successfully recruited seedling between the spring seed bag and fall seed dispersal methods. For the purpose of cost comparison between methods, site selection and water quality monitoring costs were not included.

Results

Site Selection

Based on the MD-DNR SAV Restoration Targeting System, five sites in the lower Patuxent River, Parrans Hollow (38° 24.714’ N 76° 31.649’ W), Jefferson Patterson Park (38° 24.438’ N 76° 31.268’ W), Myrtle Point (38° 19.755’ N 76° 29.493’ W), Hungerford Creek (38° 20.975’ N 76° 28.317’ W), and Solomons Island (38° 18.898’ N 76° 27.252’ W) were identified as suitable habitats for eelgrass recolonization.

Seed Collection

<u>2003:</u> Reproductive shoots from healthy eelgrass beds containing mature seeds were collected manually in Tangier Sound. Harvesting took place on May 20, 23, and 27-30 and yielded 2.3 million seeds, 250,000 of which were viable for broadcast (Table 4).

<u>2004:</u> A mechanical harvest boat was utilized to increase the efficiency and amount of reproductive material collected. From May 24 to June 4, 2004, seeds were collected from donor beds in the Little Annemessex River. In nine cutting days the mechanical harvester collected reproductive material resulting in approximately 71.92 L of eelgrass seeds. The portion of reproductive material transported to Piney Point for seed extraction yielded 15.12 million seeds (Table 4). After the seed processing and storage process was complete, 7% of the collected seeds (or 1,058,400 seeds) were viable for broadcast.

2005: Reproductive material was harvested from the Little Annemessex River and the mouth of Acre Creek (Big Annemessex River) from May 23 to June 8, 2005. The harvest machine collected approximately 109.5 L of eelgrass seeds from 21.6 acres of eelgrass beds. Seed count estimates were made after all of the seeds had fallen from the reproductive shoots and had been separated from the decaying reproductive material. Replicate 2 ml samples of seed material were analyzed for the number of viable seeds of the total number of seeds. The total number of seeds harvested was calculated as the sum of the number of seeds per ml (113/ml) and the total volume of seeds collected (109.5 L). Based on this calculation, the portion of reproductive material transported to Piney Point for seed extraction yielded 12,373,500 seeds (Table 4). An estimate of the number of viable seeds was also determined as the sum of the number of viable seeds (68 viable seeds/ml) and the total volume of seeds. Using this calculation, there were an estimated 7,446,000 viable seeds, 60% of the total number of seeds collected, after processing was through. After storage of the seeds throughout the summer, there were a total of 2,527,000 viable seeds (20% of the total number of seeds collected).

In order to estimate the number of seeds disbursed through the seed bag method in 2004 seeds were enumerated in four 1 L replicate subsamples of reproductive material shortly after collection, but before seed bags were constructed. Because a known amount of seed material was used to fill each seed bag, the number of seeds per liter, the number of seeds/seed bag, and the number of seed bags /plot could be used to calculate the total number of seeds dispersed at each location using this method (number of seeds/acre reported in Table 1).

Test Plantings

Test plantings were planted in fall of 2004 to ensure that areas identified by the site selection model would support growth of eelgrass. Adult eelgrass plants were transplanted into three 1 m^2 test plots located adjacent to seed broadcast or seed bag areas. Test plantings occurred on November 13, 2003, November 18, 2004, and November 1, 2005. A density of 64 adult plants per m^2 was used for each test plot. When the test plots planted in 2004 were surveyed in the 2005, 77% of the plants among the three test plots had survived when surveyed in May, but only 15% of these plants persisted in July at Parrans Hollow (Figure 9 and Table 5). This resulted in a 77% initial planting success rate. In July, an average of 7 plants persisted, only 15% of the initial plants that succeeded. No plants were present at any of the three test plots here in November. At Hungerford Creek, 44% of the plants had survived when surveyed in May, dropping to 25% persistence in July. No plants were present at any of the three test plots in November. At Solomons Island, 82% of the initial plants survived until May, but only 4% of these plants persisted until July. No plants were present at any of the three test plot locations in November.

Eelgrass Seeding

Patuxent River Seeding Details by Year

23.75 acres at five restoration locations in the Patuxent River have been seeded since commencement of this project (Figure 10). In October of 2003, six 0.5 acre plots were seeded by broadcasting seeds manually at Jefferson Patterson Park (Table 2). In May 2004, 381 spring seed bags were deployed containing 1.91 million seeds and covering

14.5 acres (Table 1). In October 2004, 0.75 acres were seeded by broadcasting 112,500 seeds mechanically (Table 3). Seeding also took place in August of 2005 when 368,500 seeds covering 5.5 acres were broadcast mechanically (Table 3). The remainder of the results reported here are the data from the 2004 spring seed bag and fall seed broadcast efforts.

Seeding Results by Restoration Location (2005 surveys of 2004 seeding efforts)

Parrans Hollow

In 2004, a total of 887,500 eelgrass seeds were dispersed at Parrans Hollow covering 6.25 acres at three locations (Tables 2 and 3, Figure 11). In May, spring seed bags were deployed at two adjacent areas, one 5 acres and the other 1 acre, with 605,000 and 245,000 seeds, respectively (Table 6). During the fall seed broadcast (October), a total of 37,500 seeds covering 0.25 acres were broadcast. All 2004 seeding areas were surveyed in the spring and summer of 2005.

2004 Seedling Survival Data

- Fall Seed Broadcast: No eelgrass seedlings were observed in the 2004 fall seed broadcast site during the May, July, or November 2005 surveys.
- Spring Seed Bags: A total of 120 and 104 seedlings per acre were observed at 2004 spring seed bag sites 1 and 2 when surveyed in May 2005, respectively (Figure 16A and Table 7) yielding an initial planting success of 0.05 and 0.09%, respectively at these sites (Figure 16B and Table 6).
- Other Observations: During the May 2005 survey, divers noted the presence of both *Zannichellia palustris* (horned pondweed) and *Ruppia maritima* (widgeon

grass) within the test plot area adjacent to the restoration locations. In addition, there were a number of eelgrass plants flowering just outside the test plot areas. High fouling rates were evident on the plants present in the test plots. In July 2005, the adult plants in the test plots were heavily fouled and very short, but appeared green and healthy nonetheless. Horned pondweed and widgeon grass were both observed by divers during transects. Two shoots of eelgrass were observed outside of the transect area. Clam dredge scars were prevalent throughout transects across the planting sites during both the May and July 2005 surveys.

Jefferson Patterson Park

A total of 501,000 eelgrass seeds have been dispersed at Jefferson Patterson Park covering 7 acres of bottom (Table 5). Restoration activities here have included fall seed broadcast in 2003, spring seed bag deployment in 2004, and fall seed broadcast in 2005 (Figure 12 and Table 5). In 2003, 300,000 seeds were broadcast into six 0.5-acre circles. In 2004, 150,000 seeds were broadcast over 1 acre using spring seed bags. In 2005, the fall seed broadcast resulted in 201,000 seeds being broadcast over 3 acres (Table 6).

2004 Seedling Survival Data

- Fall Seed Broadcast: No eelgrass seedlings were observed at the 2003 fall seed broadcast site during the May, June, or November 2005 surveys.
- Spring Seed Bags: A total of 155 seedlings per acre were observed at the 2004 spring seed bag site when surveyed in May 2005 (Figure 16A and Table 7) yielding an initial planting success of 0.10% (Figure 16B and Table 6).

- Other Observations: During the May 2005 survey, divers noted a high density of horned pondweed and fewer widgeon grass plants along transects of the planted area. In July and November 2005, divers noted a completely bare bottom during all transects at this location.

2005 Activity The 3 acre fall seed broadcast area will be surveyed in the spring of 2006 to determine the success of the fall seed broadcast effort that took place in October 2005.

Hungerford Creek

A total of 171,500 eelgrass seeds have been dispersed at Hungerford Creek covering 2.25 acres of bottom (Table 5). Restoration activities here included fall seed broadcast in 2004 and 2005 (Figure 13 and Table 5). A total of 37,500 seeds covering 0.25 acres and 134,000 seeds covering 2 acres were broadcast in October 2004 and August 2005, respectively (Table 6).

2004 Seedling Survival Data

- Fall Seed Broadcast: No eelgrass seedlings were observed at the 2004 fall seed broadcast area during the May, July, or November 2005 surveys.
- Other Observations: During the May 2005 survey, divers noted a thriving population of horned pondweed as well as a prominent filamentous algae cover, both of which made it extremely difficult to distinguish eelgrass seedlings at this location. Flowers were present on half of the eelgrass plants located in the test plot. In addition, there were a number of eelgrass flowering plants just outside the test plot areas. In July 2005, the adult plants in the test plots were heavily fouled and very short. When divers attempted to wipe the epiphytes from the leaves,

leaves were brittle enough that they broke away from the main plant. Evidence of worms (unidentified species) and cownose rays (*Rhinoptera bonasus*) were prevalent throughout transects across the planting sites. In November 2005, divers noted a completely bare bottom during all transects at this location.

2005 Activity The 2 acre fall seed broadcast area will be surveyed in the spring of 2006 to determine the success of the seed broadcast effort that took place in October 2005.

Myrtle Point

A total of 333,500 eelgrass seeds have been dispersed at Myrtle Point covering 3 acres of bottom (Table 5). Restoration activities here included one spring seed bag deployment in 2004 and one fall seed broadcast site in 2005 (Figure 14 and Table 5). In May 2004, spring seed bags containing roughly 300,000 seeds were deployed covering 2.5 acres. In 2005, the fall seed broadcast resulted in 33,500 seeds being broadcast over 0.5 acres (Table 6).

2004 Seedling Survival Data

- Spring Seed Bags: A total of 32 seedlings per acre were observed at the 2004 spring seed bag site when surveyed in May 2005 (Figure 16A and Table 7) yielding an initial planting success at this site of 0.03% (Figure 16B).
- Other Observations: During the May 2005 survey, divers noted an extremely strong current moving across the planted area. In July and November 2005, divers noted a completely bare bottom during all transects at this location.

2005 Activity The 0.5 acre fall seed broadcast area will be surveyed in the spring of 2006 to determine the success of the seed broadcast effort that took place in October 2005.

Solomons Island

A total of 642,500 eelgrass seeds have been dispersed at Solomons Island covering 5.25 acres of bottom (Table 5). In May 2004, spring seed bags containing roughly 605,000 seeds were deployed covering 5 acres (Figure 15 & Table 6). In October 2004, the fall seed broadcast resulted in 37,500 seeds being broadcast over 0.25 acres (Table 5).

2004 Seedling Survival Data

- Spring Seed Bags: No eelgrass seedlings were observed at the 2004 spring seed bag (Table 7) or fall seed broadcast areas during the May, July, or November 2005 surveys.
- During the May 2005survey, divers noted epiphytic growth on the adult test plot plants. Little to no vegetation was present throughout transects, and a distinct ripple pattern was evident in the sediment at the bottom. In July 2005, divers noted a completely bare bottom during all transects at this location. During all of the surveys (May, July, November 2005), divers noted an extremely strong current moving across the planted area.

Water Quality

The SAV Strategy calls not only for large-scale SAV restoration projects, but also for coincident assessment of the associated habitat conditions in order to evaluate reasons for success or failure and, in turn, improve the likelihood of success of future projects. In keeping with this requirement of the Strategy, spatially and temporally intensive water quality monitoring was conducted during 2003, 2004, and 2005.

Continuous Monitoring (temporally intensive monitoring)

Two continuous monitoring (YSI 6600 EDS) stations were located on the Patuxent River prior to and during restoration (2003-2005) to provide temporally intensive habitat assessments to complement the monthly water quality mapping. The first monitoring station was located at the Pin Oak Farm (38° 24.528’ N 76° 31.308’ W) near the Jefferson Patterson Park restoration site (MLW, 1.2 m) and the second monitoring station was at the Chesapeake Biological Laboratory (CBL) dock (38° 19.002’ N 76° 27.156’ W) near the Solomons Island restoration site (MLW, 2.4 m). Both meters were located at a constant depth of approximately one meter below the surface of the water

CBL Figure 17 includes turbidity and temperature data at the CBL continuous monitoring station for 2003, 2004, and 2005. DATAFLOW and Patuxent River mainstem cruises are marked on the graphs. The red line on the graph indicates a turbidity of 5.38, the water quality target for SAV in the Patuxent River that corresponds to 22% light to a depth of 1 m. On the 2005 panel the dates of DNR’s SCUBA surveys are also marked (May 17, July 26, and November 4).

Turbidity was lower in 2003 than 2004 and 2005, and only exceeded the 5.38 NTU limit 4.5% of the year (Table 8). Turbidity during the SAV growing season (March-October, where data available) exceeded the limit 4.6% of the time. Increased turbidity was seen from March until October 2004 with peaks between May and July. Turbidity levels exceeded limits 18.0% of the year and 20.1% of the SAV growing season. Elevated turbidity levels occurred throughout the year in 2005. The 2005 continuous monitor dataset did not start until April 6 and ended Oct 31 therefore the full year dataset available is identical to the SAV growing season dataset. Turbidity exceeded limits 13.2% of the year/SAV growing season. Between the May 17 and July 26 survey dates, turbidity exceeded the 5.38 NTU limit 24.8% of the time.

Looking at potential temperature effects during this study, thresholds for eelgrass survival of 25^{o}C and 30^{o}C were examined (Table 8). According to the continuous monitor data temperatures exceeded 25^{o}C for some period of time in every year, with the highest percentage being in 2005 (more than in 2003 and 2004), nearly 50% of the time. Temperature did not exceed 30^{o}C during 2003 or 2004. Temperature values exceeded 30^{o}C 1.6% of the time during the SAV growing season and 0.3% of the time between surveys in 2005.

Pin Oak Farm Figure 18 includes turbidity and temperature data at the Pin Oak continuous monitoring station for 2003, 2004, and 2005. DATAFLOW and Patuxent River mainstem cruises are marked on the graphs. The red line on the graph indicates an NTU of 5.38, the water quality target for SAV in the Patuxent River that corresponds to 22% light to a depth of 1 m. On the 2005 panel the dates of DNR's SCUBA surveys are also marked (May 17, July 26, and November 4).

Turbidity values were higher at the Pin Oak station than the CBL station across all three years. Despite being lower in 2003 than 2004 and 2005, turbidity exceeded the 5.38

NTU limit 41.9% of the year (Table 8). Turbidity during the SAV growing season (March-October, where data available) exceeded the limit 45.6% of the time. Increased turbidity was seen throughout the year in 2004. Turbidity levels exceeded limits 64.6% of the year and 70.9% of the SAV growing season. Elevated turbidity levels occurred throughout the year again in 2005. The 2005 continuous monitor dataset did not commence until April 6 and ended Oct 31 therefore the full year dataset available is identical to the SAV growing season dataset. Turbidity exceeded limits 54.7% of the year/SAV growing season. Between the May 17 and July 26 survey dates, turbidity exceeded the 5.38 NTU limit 62.7% of the time.

At the Pin Oak station, temperatures were above 25^{o}C over 60% of the year and growing season in 2003, dropped slightly in 2004 (38.2 and 42.8% during the year and growing season, respectively), and were high again in 2005 exceeding 25^{o}C over 50% of the time for the entire dataset/growing season and 61.3% of the time between the May 17 and July 26 surveys (Table 8). Temperature exceeded 30^{o}C 3.7% of the year and 4.0% of the SAV growing season in 2003. Temperature only exceeded 30^{o}C 0.5% of the year and 0.6% of the SAV growing season during 2004. In 2005, temperature values exceeded 30^{o}C 8.0% of the time during the SAV growing season and 9.1% of the time between surveys.

Fluorescence (Chla) was another parameter monitored by the continuous monitor stations. Correlation values were determined between turbidity and Chla values from the 2003, 2004, and 2005 continuous monitor datasets. At the CBL station in 2003 the

Pearson correlation coefficient was 0.5 ($P < 0.0001$, $N = 11905$). The 2004 and 2005 correlations yielded coefficients of 0.03 ($P < 0.0001$, $N = 25876$) and 0.14 ($P < 0.0001$, $N = 17423$), respectively. At the Pin Oak station in 2003 the correlation coefficient was 0.48 ($P < 0.0001$, $N = 10637$). The 2004 and 2005 correlations yielded coefficients of 0.03 ($P < 0.0001$, $N = 25323$) and 0.14 ($P < 0.0001$, $N = 16564$), respectively.

DATAFLOW (spatially intensive monitoring)

Spatially intensive water quality monitoring (water quality mapping) was conducted monthly throughout the eelgrass-growing season (March – November) in the lower portion of the river utilizing MD-DNR DATAFLOW systems. Turbidity data from the Patuxent River DATAFLOW cruises were complied and analyzed for 2003, 2004, and 2005.

DATAFLOW cruises were conducted and turbidity data analyzed from April through October 2003 (Figure 19), March through November 2004 (Figure 20), and April through September 2005 (Figure 21). In 2003, turbidity peaked between May and June throughout the river (Figure 19). Despite remaining high in the upper reaches of the river until July, turbidity decreased in the areas where restoration was being conducted in July. High turbidity events seemed to be spatially patchy in 2004 (Figure 20). In 2005, peaks in turbidity were evident in the upper river from April through July (Figure 21). Turbidity in the lower river near the restoration locations appears to have remained constant between 2.5 and 5 NTU’s until August when it decreased.

Secchi depth (Figure 22), TSS (Figure 23), and temperature (Figure 24) values for 2003, 2004, and 2005 (secchi depth and temperature only) were compared to the range and mean of available data from 1985 until 2002 at Saint Leonard, Point Patience, and Drum Point. Water clarity values for 2003, 2004, and 2005 are close to the mean, and do not fall outside of the range when compared to the 20-year record available at these three stations on the Patuxent River. In 2005, temperatures were uncharacteristically high, falling outside of the 20-year temperature range for the months of August and September at each of the three stations, and October at Point Patience.

Cost Comparison and Survival Calculations

At the conclusion of the first year of restoration, several cost calculations were made based on the planting results. To determine the financial investment made per seed dispersed, the total cost of the particular method was divided by the total number of viable seeds dispersed using that method.

The cost per seed put out on the Patuxent River (ignoring survival) $0.02 for the spring seed bag method and $0.34 for the fall seed broadcast. The total cost for restoring one acre was determined by multiplying the cost per seed by the specified seeding density (200,000 seeds/acre for both methods). The cost for restoring one acre was determined to be $4,473 for the spring seed bag method and $67,085 for the fall seed broadcast method.

The recruitment success of each method was determined by dividing the total number of seeds dispersed by the number of successfully recruited plants. The spring seed bag method yielded 874 seedlings across all spring seed bag sites locations in May 2005. A total of 1,910,000 seeds were dispersed using this method. Therefore the overall recruitment success for the spring seed bag method was 0.05% after 11 months. However, none of these plants survived the summer. The fall seed broadcast method did not yield any seedlings. Therefore, regardless of the number of seeds broadcast this way; the recruitment success of that method was 0.0%.

The total cost for each method was divided by the total number of successfully recruited seedlings to determine cost per successfully recruited seedling between the spring seed bag and fall seed dispersal methods. Each seedling (874) successfully recruited using the spring seed bag method cost $11.15. This figure could not be calculated for the fall seed broadcast method due to the lack of successful recruitment.

For the purpose of cost comparison between methods, site selection, monitoring, and certain salary costs were not included. A more comprehensive cost analysis of the above-cited figures including site selection, monitoring and salary costs will be included in the final project report.

Despite the low seedling survival, much useful information came out of this project which will be discussed below.

Project Website

In addition to field work and other requirements of this project, Maryland Department of Natural Resources created an online website resource specific to this project (http://www.dnr.state.md.us/bay/sav/restoration/pax_gen_info.asp). Included in this webpage is background information about this project, a timeline of major project activities, maps depicting restoration locations, explanation of project methods, seeding details, and photographs of the various stages of this project. This and subsequent reports will be posted on the website in PDF format once complete.

According to a WebTrends report produced by MD-DNR Information Technology Services, 4,619 visitors have logged a total of 26,987 successful hits, an average of 146 hits per day since it was posted in late July. Visitors spent an average of 11 minutes navigating the website. The Patuxent River Eelgrass Restoration Project page (http://www.dnr.state.md.us/bay/sav/restoration/pax_gen_info.asp) ranked as the third most popular webpage visited from the restoration homepage (address above).

Discussion

Early eelgrass restoration efforts on the Chesapeake Bay involved transplanting adult eelgrass plants from healthy source beds to restoration locations with costs averaging $37,000/acre excluding monitoring costs (Fonseca et al. 1998). This and other restoration methods are both expensive and labor intensive, and can damage donor beds. Despite some advantages to using adult plants, for example, successful adult plants yield reproductive shoots during the following year's reproductive season (Orth et al. 2003), broadcasting seed appears to be a more efficient and cost effective restoration technique with the added benefit of having less impact on donor beds (Orth et al. 2000). In order to meet the goals of the CBP and the SAV Strategy, MD-DNR developed this project to conduct large scale eelgrass restoration using seeds at select locations on the Patuxent River, Maryland.

Site Selection

The most important step of any restoration project is selecting the proper location. Poor site selection has been identified as a major limitation in restoration project success (Harrison 1987; Fonseca 1992). Locations have typically been based as much on logistics and practicality as on data from habitat assessments that indicate suitable conditions for SAV success. At the start of this project, much attention was given to site selection. This included the development and refinement of a modeling program designed specifically to assess large areas of Chesapeake Bay for their restoration potential. Following computer identification, the sites identified underwent a two-year site selection process of test plantings and water quality monitoring. The Patuxent River was one such location.

The Patuxent River is one of the most monitored and modeled rivers of its size in the world. It has become an important proving ground for many of the Chesapeake Bay Program Initiatives (Maryland Department of Natural Resources 2005). Prior to the decline of SAV beds in Chesapeake Bay in the 1960's and 1970's, the Patuxent River supported diverse populations of SAV including *Zannichellia palistris*, *Ruppia maritima*, *Potamogeton perfoliatus* and *Zostera marina* (Brush and Davis 1984). Eelgrass was documented in the lower Patuxent, southwest of Solomons Island, during a 1971 ground survey (Peter Bergstrom, personal communication). The combination of documented historical eelgrass coverage, water quality meeting the SAV habitat requirements according to the SAV targeting system (Parham and Karrh 1998), and the vast water quality dataset for the Patuxent River made this river a prime candidate for large scale eelgrass restoration efforts.

Seed Collection

Harvesting of eelgrass seeds for restoration in Chesapeake Bay involved hand collection using SCUBA or snorkeling through 2003. This can be effective for small-scale restoration, but to meet the Chesapeake 2000 goal of 1,000 acres by 2008, innovative techniques for enhanced seed collection were needed. Seed collection was increased from approximately 2.3 million seeds in 2003 using hand harvesting, to approximately

15.1 and 12 million seeds in 2004 and 2005, respectively. This was due primarily to the use of a mechanical harvester in 2004 and 2005.

Sexual reproduction of eelgrass occurs when adult plants produce a reproductive shoot that extends up into the water column above the plant. When using the mechanical harvester to collect reproductive material in large quantities, a number of steps were taken to prevent harming the existing plants within the donor beds. The depth of the cutting blades on the harvester was adjusted to avoid damage to the rhizome mat and lower vegetative parts of the eelgrass plants. This prevents damage to a bed because the flowering shoots are adapted to break off and be carried away by wind and tides for the dispersal of the seeds carried within. Leaving the rhizome mat intact with some amount of vegetative material still attached allowed plants to continue to grow after cutting was performed. After the seeds fall out of the reproductive shoots, these shoots serve no further purpose in the health or continued success of the bed (Granger et al. 2002). As a precaution, harvesting took place over a large area to assure that sufficient seeds remained for bed maintenance (Granger et al. 2002). To confirm that there was no significant damage to eelgrass beds where reproductive shoots had been harvested, divers used SCUBA to survey the harvested beds 8 weeks (July 22, 2004) after the 2004 collection. Divers reported abundant, healthy eelgrass and quite a bit of flowering widgeon grass. There were no substantial differences in plant height, bed density, or apparent vigor of the plants themselves between the harvested and unharvested beds. In addition, aerial photography taken on June 19 and July 6, 2004 confirmed that the areas that were harvested in May were still densely vegetated (VIMS; 2004 field observations and aerial photography accessible:

http://www.vims.edu/bio/sav/2004obs.html#vims071304).

Test Plantings

Test plantings were carried out to ensure that areas identified by the site selection model would support eelgrass growth. The plants were able to root successfully as seen by the persistence of plants from November 2004, when planted, through the winter and spring until surveyed in May 2005. The purpose of these plants is to serve as an indicator of what to expect from seedlings that emerge as a result of seedings. Seedlings would not be expected to thrive if healthy transplanted adult plants were not able to survive. The total loss of all plants at each of the test plot locations during the summer of 2005 is an indication of poor water quality during that time.

Eelgrass seeds in Restoration

Planting density effects

Restoration projects utilizing eelgrass seeds in Maryland and Virginia have been successful in the field (Orth et al. 2003). The density at which seeds are broadcast and the size and location of the plots are considered potentially important factors affecting the germination of eelgrass seeds and plant survival. Orth et al. (2003) tested five seeding densities ranging from approximately 10,000 seeds/acre to 5,000,000 seeds/acre at twelve different location baywide (both MD and VA) and found no density dependent effects on germination rate or seedling success. In the Patuxent, during the current study, seeds were broadcast at a density of 150,000 seeds/acre in 2004. Initial seed broadcasts in

August of 2005 were carried out at a density of 67,000 seeds/acre. Since the data for this report was prepared, these same areas have since been enhanced with an additional 200,000 seeds/acre resulting in a final seed density of 267,000 seeds/acre for all 2005 broadcasts. After spring surveys (May 2006) the effectiveness of different seeding densities will be closely examined to evaluate the potential for site-specific variation in density dependence.

Plot Size Effects

Some literature suggests that larger, denser, restored beds are more likely to positively influence water quality, and therefore, are less susceptible to perturbations such as storms or mute swans. Due to the physical presence of three-dimensional structure provided by SAV, and the increased "roughness" of the bottom in SAV beds, water velocities are reduced as much as 50% reduced within SAV beds (Fonseca et al. 1982; Benoy and Kalff 1999; Gacia et al. 1999). Furthermore, it has been noted that water velocity reductions are directly proportional (as a power function) to both the height and the growth form of the species that occur in the area (Petticrew and Kalff 1992; Gacia et al. 1999). However, despite reasons to expect that plot size might affect plant survival, restoration efforts with eelgrass in plots of different sizes (4 m^2 to 400m^2) and configurations (alternating 4 m^2 patches and large continuous patches) in different river systems in Virginia (Virginia Institute of Marine Science) have shown a significant site effect but no significant plot size effect on germination of eelgrass seeds (Orth, personal communication).

Seed Storage Issues

Storing the spring-harvested seeds over the summer is one of the most difficult aspects of this project. Each year there has been a substantial loss of seeds during seed storage, ultimately decreasing the number of viable seeds at the end of the storage process and reducing the acreage of SAV restored for this project. During the seed harvest of 2003, 2.3 million seeds were collected, only 250,000 (11%) of which were viable and used for broadcast. Harvest efforts in 2004 collected 15.12 million seeds. However, 1,058,400 seeds (7%) of these were deemed viable in the fall. The 2005 harvest collected 109.5 liters, 12,373,500 seeds. Unfortunately, only 2,527,000 (20%) seeds were viable at the end of the seed processing/storage procedure. After the 2004 season, biologists from VIMS and DNR attempted to identify potential problems with the seed transport and separation and holding/storage process. The lack of general research on seed physiology made identifying specific problems very difficult. In 2005, seed storage experiments were set up at St. Mary's College, VIMS, and MD-DNR to test the impact of the following parameters: flow, aeration, salinity, and stirring. When the results of these experiments are analyzed, appropriate modifications will be made to the seed processing and storage procedure to be applied in 2006.

A buoy-deployed seeding system (BuDSS) developed by Pickerell et al. (2003; 2005) was modified slightly and used as an alternative method to broadcast seeding in the fall. There are several potential advantages to using this method, most importantly, eliminating the need to store seeds during the summer. For this method, reproductive material is placed in mesh bags immediately after harvest, moved to the restoration location, and deployed in the area to be restored. Immediate deployment of reproductive material eliminates the need to store seeds, reducing the number of seeds lost to processing and decreasing the expense and labor requirements associated with seed transport, processing, and storage. The mesh bags remain suspended at the top of the water column, allowing the seeds to develop and drop over a period of weeks. This mimics the floating and rafting of reproductive shoots during natural seeding events during the natural phenological schedule (Pickerel et al. 2003; 2005). Although not proven, it has been suggested that this method may also reduce seed predation by spreading out seed dispersal over time and through a combination of time and natural forces yield a more even distribution of seeds. There are also potential problems with this method. These bags create a navigational hazard while the mesh bags are on-site (the restoration plots with floats every 10 meters are difficult to navigate). Despite staggering seed dispersal over time, seed predators are active during this time. Any sort of spring dispersal that mimics the natural dispersal will be affected by predators. In addition, the dispersal of seeds from the bags is not random and follows a figure eight shaped pattern following the ebb and flood tides as seen by Pickerel (2003).

Determining Viability

One difficulty in using eelgrass seeds for restoration lies in determining the number of viable seeds as a proportion of total seeds being put out. Whether for use in fall seed broadcasts or spring seed bags, it is necessary to know the number of viable seeds in order to determine the recruitment rate (number of seedlings/number of viable seeds put out).

Two methods were used to count seeds, one for the spring seed bag method and one for fall seed broadcast method. At the present time there is no way to know exactly how many viable seeds are broadcast by the spring seed bag method. The number of seeds put out in seed bags was estimated by counting seeds in four 1L replicate subsamples of reproductive material and multiplying the resulting seeds/L by the total volume of harvested material used in the construction of the seed bags. This gives us an estimate of the total number of seeds dispersed using the seed bag method. However, once the bags are deployed there is no way to collect spathes from the bags once the seeds are mature and begin falling out. Seeds are never extracted directly from the spathes to analyze each of them individually and therefore, there is no direct measure of the number of viable seeds vs. dead or non-viable seeds. Therefore recruitment is calculated from the number of seedlings recruited/the total number of seeds dispersed. A method to estimate the number of viable seeds released from spring seed bags is in the process of being investigated for application during the 2006 season.

The seed estimate for the fall seed broadcast method was made after all of the seeds had fallen from the reproductive shoots and were separated from the decaying reproductive material. This method also has some inherent sources of uncertainty. Because good seeds separate from bad seeds in water, it is necessary to drain all of the water from the seed slurry and completely mix the seed mixture before obtaining a representative sample. In addition, human error is a factor in both measuring samples out as well as the squeeze test for viability. When measuring aliquots, seeds are very sensitive to packing, creating a lot of variability between the 2 ml samples. During the squeeze test a seed is deemed viable or not viable based on physical robustness of the seed. There is considerable subjectivity in this determination as well. Efforts were made to keep the methods as uniform as possible, but because of the vast number of counts that are made it is not feasible to use the same staff member to conduct all counts. We have not been able to determine to what degree these sources of error affect our estimates.

Eelgrass Seeding Success

None of the fall seed broadcast sites on the Patuxent River successfully recruited eelgrass seedlings. Because seeds from the same batch that were broadcast on the Potomac River, MD and at several sites in Virginia (VIMS) successfully recruited plants, it can be assumed that the seeds broadcast were indeed viable. At the Parrans Hollow and Solomons Island locations, seeds were successfully recruited in the spring seed bag plots suggesting that conditions at these locations were appropriate for seed recruitment. There was not a spring seed bag plot at Hungerford Creek, and without some means to gauge whether or not eelgrass seeds were capable of being successfully recruited, this may have been an inappropriate site for eelgrass restoration. However, this site supported the adult eelgrass plants in the test plots.

The seed bag method was successful at all locations except Solomons Island. Seed deployment through the spring seed bag method yielded an average recruitment rate of 0.046%. When reviewed on a site by site basis, recruitment rates were higher at the northernmost restoration locations than at the sites closer to the mouth of the river. There was no recruitment of seedlings at Solomons Island. Recruitment increased upriver to 0.3% at Myrtle Point, and 0.05 and 0.09% at the Parrans Hollow locations. Recruitment was highest, 0.10%, at the Jefferson Patterson Park location.

Eelgrass seed recruitment as a percentage of total seeds during natural seeding appears to always be quite low. Annual seed production ranges from 6,176 seeds/m^2 to 24,460 seeds/m^2 (Olsen 1999). However, reported seedling numbers are significantly less than the numbers of seeds produced, ranging from 5-15% of the seeds produced (Harper et al. 1965; Cook 1979; Olsen and Sand-Jensen 1994; Cabin et al. 2000; Granger et al. 2002; Orth in press). Unfortunately, our seedling recruitment results were far below this average. Researchers using seeds in experimental plantings have encountered varied success as reported above, but common outcomes include, low germination rates (Moore et al 1993), wash-out of seeds (Orth et al. 1994, Harwell and Orth 1999), and seed predation (Fishman and Orth 1996).

Germination of eelgrass seeds in Chesapeake Bay is thought to be dependent upon temperature, burial, and oxygen cues (Orth and Moore 1983; Moore et al. 1993). Incorporation of seeds into the sediments (Orth and Moore 1983; Moore et al. 1993) is essential for the start of germination. Eelgrass seeds are rapidly incorporated into most sediments and generally do not move far for where they settle under various hydrodynamic regimes (Orth et al 1994). The complexity of the bottom due to biological and physical processes appears to be important for seed retention (Luckenbach and Orth 1999). Orth et al. (1994) demonstrated that turbation of the sediment as little as 1 millimeter deep could stop an eelgrass seed from rolling and being transported away. However, deep burial can stop germination. Burial of seeds below the redox potential discontinuity prevents the developing plant from receiving light (Bigeley 1981) which may be crucial to germination.

Although not made before the seedings took place, observations by divers during the 2005 surveys suggested that the bottom at Parrans Hollow and Jefferson Patterson Park were suitable for seed recruitment. Hungerford Creek showed signs of worm populations that could increase bottom roughness for settlement of seeds. Through burrowing and bioturbation, benthic infaunal species can increase seed retention through the formation of micro-sites (Rhoads and Young 1970; Rhoads 1974). A very strong current was present causing a distinct rippling pattern in bottom sediments at Myrtle Point and Solomons Island that could act to entrain seeds.

Although a strong current causes texture in the bottom that may enhance seed recruitment, it may also act to wash out or transport seeds from the dispersal site preventing germination (Chambers and MacMahon 1994; Orth et al 1994; Harwell and Orth 1999) and even uproot new seedlings or adult plants. Although we did not measure seed predation it also appears to be an important factor in seed loss (Janzen 1971; Wassenberg 1990; Fishman and Orth 1996). However, without examining these issues closely during this project, it is difficult to base conclusions on this information.

Eelgrass Survival Related to Water Quality

Perhaps more important to this project than the low recruitment rates is the inability of the recruited seedlings to survive the summer. As we saw in May 2005, seedlings were successfully established at all sites except Solomons Island, so we know that the use of the seed bag method on the Patuxent River has the potential for successful restoration.

However, both adult test plots and seedlings completely disappeared in the summer of 2005. To determine the cause for this, the water quality data collected during this study were evaluated.

Light attenuation occurring through the water column and at the leaf surface appears to be the most important factor effecting SAV growth (Kemp et al. 1983; Wetzel and Penhale 1983; Dennison 1987). A number of studies have shown that decreased light availability affects the survival of eelgrass in particular (Wetzel and Hough 1973; Phillips et al. 1978; Kemp et al. 1983; Twilley et al. 1985; Dennison and Albert 1986). Light availability data from the continuous monitoring stations, DATAFLOW cruises, and long term fixed-station cruises were analyzed to detect trends or spikes that may help explain the lack of survival of seedlings.

Turbidity values are one measure we have to determine light availability in the Patuxent during our study period. Using the EPA requirement of 22% of surface irradiance for healthy mesohaline species SAV growth, and an application depth of 1 m, a turbidity value of 5.38 NTU was determined as the water clarity target for the Patuxent River. When we look at the percent of the time that turbidity values exceed this limit, the difference between data at the Pin Oak Farm station and the CBL station are marked. Turbidity exceeded this limit significantly more at the Pin Oak Station, 46%, 71% and 63% of the growing season in 2003, 2004, and 2005, respectively compared to 5%, 20%, and 25% at CBL. At both stations, 2004 and 2005 conditions were worse than 2003, with 2005 being a slightly better year than 2004.

In addition to attenuation of light through the water column, epiphytic growth on the blades of SAV can further reduce light availability (Borum 1985; Twilley et al. 1985; Burt et al. 1995). On the Patuxent River, Stankelis (2003) found the highest fouling rates at the least turbid sites downriver and the lowest fouling rates at the most turbid sites, upriver. Although turbidity was significantly less near to the mouth of the river at the Solomons Island site, this decreased turbidity may have allowed for increased epiphyte growth causing the death of the adult plants there. The persistence of the adult test plot at this site as well as the upriver site and their simultaneous die off, however, is not consistent with these observations.

The data reported here reflect the results of 2004 seeding efforts. Germination of these seeds took place in the fall of 2004. New seedlings recruited from 2004 seeding efforts were subject to 2005 spring water quality conditions. Keeping in mind the importance of light, when we look closely at the turbidity conditions occurring between our three surveys, it is evident that there were episodes of severely elevated turbidity at both of the continuous monitoring stations. At the Pin Oak station there was an extended period of time, June 29^{th} until July 17^{th} 2005 when turbidity exceeded the habitat requirement. This episode ended just one week before our July 27^{th} survey. Eelgrass needs between 6 and 8 hours of photosynthetic saturating irradiance to survive (Dennison and Alberte 1986). Although it is not well documented how many days healthy plants can survive elevated turbidity and decreased light availability, it is not likely that the recruited seedlings or adult plants could survive the prolonged periods of high turbidity such as those reflected by the continuous monitor data.

Although the DATAFLOW data present a snapshot of water quality for one given time, it is useful for comparing water quality in different regions of the river. The 2005 DATAFLOW data showed recurring elevated turbidity near the Pin Oak Farm continuous monitor location, close to the Parrans Hollow and Jefferson Patterson Park sites from June to September 2005. This information coincides with the poor water quality data from the continuous monitor station near these locations.

Light attenuation in the water column (turbidity) is strongly affected by both total suspended solids and (TSS) and chlorophyll a (Chla) in the Cheseapeake Bay region (Batiuk 2002). Chla was measured and recorded by continuous monitors however, TSS data was not. In 2003, correlations between Chla and turbidity were 0.5 ($P < 0.0001$, $N = 11905$) at the CBL station and 0.48 ($P < 0.0001$, $N = 10637$) at the Pin Oak Station suggesting that changes in turbidity were due in part to changes in Chla concentrations. Both of these correlations are much higher than the values at either station in 2004 and 2005. Chla and turbidity showed very weak correlations at both stations in those years suggesting that the changes in turbidity seen in 2004 and 2005 are not likely attributable to Chla. These weak correlations suggest that suspended solids, not Chla, may be the cause of the high turbidity in the Patuxent River. However, without TSS data being collected by the continuous monitors for this time frame, we can not definitely conclude that the increased turbidity is caused by TSS.

Fixed station, monthly, water quality monitoring cruises have been conducted at eleven mid-channel stations throughout the mainstem of the Patuxent River since 1985. Both secchi depth and TSS values for 2003, 2004, and 2005 (secchi depth only), were compared to the range and mean of available data from 1985 until 2002 at Saint Leonard, Point Patience, and Drum Point. Water clarity values for 2003, 2004, and 2005 are close to the mean, and do not fall outside of the range when compared to the 20-year record available at these three stations on the Patuxent River.

In Chesapeake Bay, eelgrass is near the southernmost reaches of its distribution on the east coast of the United States. There is a well documented bimodal eelgrass growth pattern with maximum growth and a peak in biomass occurring in late May to early June. A second, less dramatic growing season occurs in mid-September and continues until water temperatures drop below 10^{o}C sometime in November. Increasing light attenuation and water temperature (above 25^{o}C) later in June cause decreased growth and leaf defoliation (Moore et al. 1996; 1997). The metabolic rates of eelgrass are directly affected by temperature, and if too high, can increase plant respiration high enough to kill the plant (Thom et al. 2001).

Our data are consistent with this pattern. Seedlings were present and the test plots plants were thriving in mid-May 2005, but the majority of the seedlings as well as the test plots were absent when the same areas were surveyed in July 2005. If conditions were ideal and the plants had simply undergone a summer defoliation, we would have expected to have seen those plants again during the November survey during the fall growing period. This was not the case suggesting that the plants died rather than underwent a seasonal defoliation. Subsequent surveys of areas devoid of plants could include examination of sediment for the presence or absence of intact rhizome systems in order to make better conclusions as to the fate of recruited seedlings.

Looking at potential temperature effects during this study, thresholds for eelgrass survival of 25^{o}C and 30^{o}C were examined. According to the continuous monitor data at both stations, temperatures exceeded 25^{o}C at both station in every year. At the CBL station, temperature exceeded 25^{o}C more in 2005 than in 2003 and 2004, nearly 50% of the time. At the Pin Oak station, temperatures were above 25^{o}C over 60% of the time in 2003, dropped in 2004, and were high again in 2005 exceeding 25^{o}C over 50% of the time. According to the continuous monitor data at both stations, temperatures exceeded 30^{o}C for some amount of time in all years between June and Mid-September. In 2005 at the Pin Oak Station, temperature exceeded this limit consistently throughout from mid-July through September. Although it is not well documented how many days healthy plants can survive elevated temperatures, the fact that these instances of elevated temperature coincide with elevated turbidity events makes it unlikely that the recruited seedlings or adult plants could survive.

Temperature values for 2003, 2004, and 2005 were compared to the range and mean of available data from 1985 until 2002 at Saint Leonard, Point Patience, and Drum Point. Temperatures for 2003 and 2004 are close to the mean, and do not fall outside of the range when compared to the 20-year record available at these three stations on the Patuxent River. However, in 2005, temperatures were uncharacteristically high, falling outside of the 20-year temperature range for the months of August and September at each of the three stations, and October at Point Patience.

Cost Comparison Calculations

In an effort to find the most cost-effective restoration method, attempts were made to determine the financial investment made on a per-seed basis. To do this, the total cost of the particular method was divided by the total number of viable seeds dispersed using that method. The cost per seed put out in Maryland was $0.02 for the spring seed bag method and $0.34 for the fall seed broadcast. The total cost for restoring one acre was determined by multiplying the cost per seed by the specified seeding density (200,000 seeds/acre). The cost for restoring one acre was determined to be $4,473 for the spring seed bag method and $67,085 for the fall seed broadcast method. The large seed loss during storage (93%) is responsible for the significantly higher costs per seed and per acre using the fall seed broadcast method. If 50% of the total seeds had been retained throughout the processing and storage procedure, a total of 803,628 viable seeds would have been available for broadcast on the Patuxent River, 10.69% of total (the rest was allocated for use in Potomac River, MD and VIMS). With 803,628 seeds and the same total cost for broadcast, the cost per seed would be reduced from $0.34 to $0.05 dropping the cost per acre from $67,085 to $9,379.55. This is not an unreasonable expectation as VIMS retained 80% of total seeds as viable in 2005 (Orth personal communication).

In order to achieve the same seed cost ratio as the seed bag method ($0.02/seed and $4,473/acre), 5,620,000 seeds would have to broadcast on the Patuxent River, 37% of the total 15.12 million seeds collected. In order for this to occur, seed viability throughout the storage process would have to be increased significantly, and a larger proportion of viable seeds (37%) would have to be allocated to the Patuxent River than in previous years (10.69%). It seems that the high costs of processes associated with the seed broadcast method, seed processing and storage, make it significantly more expensive than then dispersing seeds using the spring seed bag method.

The recruitment success of each method was determined by dividing the total number of seeds dispersed by the number of successfully recruited plants. As stated previously, however, since there was considerable difficulty in determining the number of viable seeds, this analysis is largely speculative. The spring seed bag method yielded 874 seedlings across all spring seed bag sites locations. An estimated total of 1,910,000 seeds were dispersed using this method. Therefore the overall recruitment success for the spring seed bag method was 0.05%. The fall seed broadcast method did not yield any seedlings and regardless of the number of seeds broadcast this way; the recruitment success of that method was 0.0%.

The total cost for each method was divided by the total number of successfully recruited seedlings to determine cost per successfully recruited seedling between the spring seed bag and fall seed dispersal methods. Each seedling (874) successfully recruited using the spring seed bag method cost $11.15. This figure could not be calculated for the fall seed broadcast method due to the lack of successful recruitment.

Other restoration efforts using the fall seed broadcast method throughout MD (DNR) and VA (VIMS) resulted in recruitment rates as ranging from 0.5 to 14.0% in 1999 and 4.3 to 13.8% in 2000. If MD DNR recruitment rates improved to be similar to these, through modification and improvements in methods, based on the 2004 cost and the number of seeds put out, the cost per successfully recruited plant would be $40.83-$1.46 according to 1999 rates and $4.75-$1.48 according to 2000 rates.

A seed bag project being conducted concurrently in 2004 in Seaside, VA (Coastal Bays; VIMS) resulted in a recruitment rate of 1.3%. Initial restoration efforts using the BuDSS in the Peconic Estuary, NY yielded 4 % recruitment (Pickerell et al. 2003). If MD-DNR recruitment rates for the seed bag method ranged from 1.3-4.0%, based on the 2004 cost and the number of seeds put out, the cost per recruited plant would drop to $0.39-$0.13. The projected cost of $0.39 per plant at a 1.3% recruitment rate would still be more cost efficient than the cost per plant using the seed broadcast with a 14% recruitment rate. Seeds were successfully recruited at the Solomons Island location in 1999 by VIMS, however no plants were recruited during the 2004 seeding. Upon comparing 1999 and 2004 seeding efforts (both fall seed broadcast and spring seed bag) at several locations, it is clear that 2004 success rates are far lower than those achieved in 1999, another indication of poor water quality in 2004. Regardless of which method is used, it is abundantly clear that we must work to improve recruitment dramatically and we must increase the density of our seeding in order to more closely mimic the density of naturally occurring eelgrass beds.

Project Website

During this year of this project, a website designated to this project was created with the hopes of educating the public about the importance of bay grasses as well as increasing awareness of current efforts to restore bay grasses in the Chesapeake Bay. The WebTrends report produced by MD-DNR Information Technology Services indicated that the website is well utilized as an educational tool. These data will be reevaluated in 2006 for long term trends in usage. The data and associated figures presented in this report will be incorporated into the website upon submission of this report. This and future reports will also be available for downloading in PDF format from the webpage. (http://www.dnr.state.md.us/bay/sav/restoration/pax_gen_info.asp)

Other Problems

One of the components of the proposed work included epiphyte strip deployment, and three types of predator exclosures at each restoration site. Epiphyte strips are mylar strips developed by Dr. Walter Boynton (University of Maryland) that provide a relative, but quantitative measure of the amount of epiphytic fouling on simulated SAV blades (Stankelis et al. 1999). Epiphyte data was collected but was not analyzed in time for inclusion in this report. The results of these experiments will be included in the 2006 final report.

Several SAV predators have been demonstrated to cause damage to or destroy small-scale SAV restoration plots throughout Chesapeake Bay, including the Patuxent River. One reason for moving to large-scale SAV restoration is that large plots may be better able to withstand a small or moderate amount of predation that would destroy smaller plots. To test the effects of predation, four levels of predator exclosure were to be tested at each site: no exclosure, mute swan exclosure (surface), cownose ray exclosure (bottom), and both mute swan and cownose ray exclosure (surface and bottom). However, because of the lack of substantial numbers of recruited seedlings, these trials were delayed until a sufficient number of seedlings are established.

Similarly, this project was to determine whether the created eelgrass beds are expanding through vegetative propagation and/or natural seeding. When a sufficient number of eelgrass seedlings are established, the seed plots and surrounding area will be surveyed in the spring and fall following seeding using aerial overflights and groundtruthing with a handheld mapping GPS.

Changes in 2005 Seeding

Because of the higher success we saw at the Parrans Hollow and Jefferson Patterson Park locations, the majority of the 2005 seed dispersal efforts were concentrated near these restoration locations. In addition, seeding density was increased to between 200,000 and 267, 000 seeds/acre. All previous efforts used between 100,000 and 245,000 seeds/acre. In 2003, Orth et al. were able to demonstrate that seed-seed interactions do not affect germination when dispersing seeds at increased densities. Thus seeding at higher densities could increase seedling production.

Continued Research and Monitoring Needs

Throughout the first two years of this project several research needs have emerged. There is a lack of general research about seed physiology. Increased knowledge about the physiology of eelgrass seeds would help to solve ongoing problems with seed processing and storage. Similarly, a more reliable and universally accepted means of determining the number of total seeds and the percent of those seeds that are viable is needed. With poor water quality continuing to be of major concern, it is important to start to compare the resiliency of plants to different light and temperature regimes. Information such as this would help us to better understand the tight coupling between temperature and light effects that now exists. Lastly, field methods for monitoring projects such as this one could be modified to include examining the sediment for rhizomes to confirm total disappearance vs. characteristic defoliation of eelgrass plants in the summer. This information would be helpful to determine whether disappearance of plants is due mainly to death or natural dieback of the above ground plant material.

Conclusions

It is clear that water quality, specifically turbidity and temperature, as well as epiphyte loading are all potential causes for the lack of success of this project thus far. Because we were able to successfully recruit eelgrass seedlings at all but one site, using spring seed bags, we are confident that our methods are suitable for utilizing the spring seed bag method for large scale restoration in Maryland. However, if we expect these seedlings to be successful and survive, we need to continue to increase water quality. In addition we are now aware of several technical issues that we can address to increase the success of our methods. We have begun to and will continue to address problems with seed processing, seed loss during storage, and seed counting.

Even with the technical problems causing a large loss of seeds, and the loss of all recruited seedlings that occurred, our understanding of the physical and chemical processes of this river as well as eelgrass plants and seeds have increased significantly. This report details a number of problems solved, essential information uncovered, as well as changes made for future harvest/storage/broadcasts.

It is important that we keep in mind that we expect year to year variation in eelgrass population size and growing conditions, and therefore varied success of new seedlings. With this in mind, it is still likely that if significant numbers of plants can be established in dense, protected beds, the combination of physical protection and the benefits self-protection may enable the establishment of substantial areas of eelgrass habitat within the Patuxent River.

Appendix A

Basin Water Quality Monitoring Results

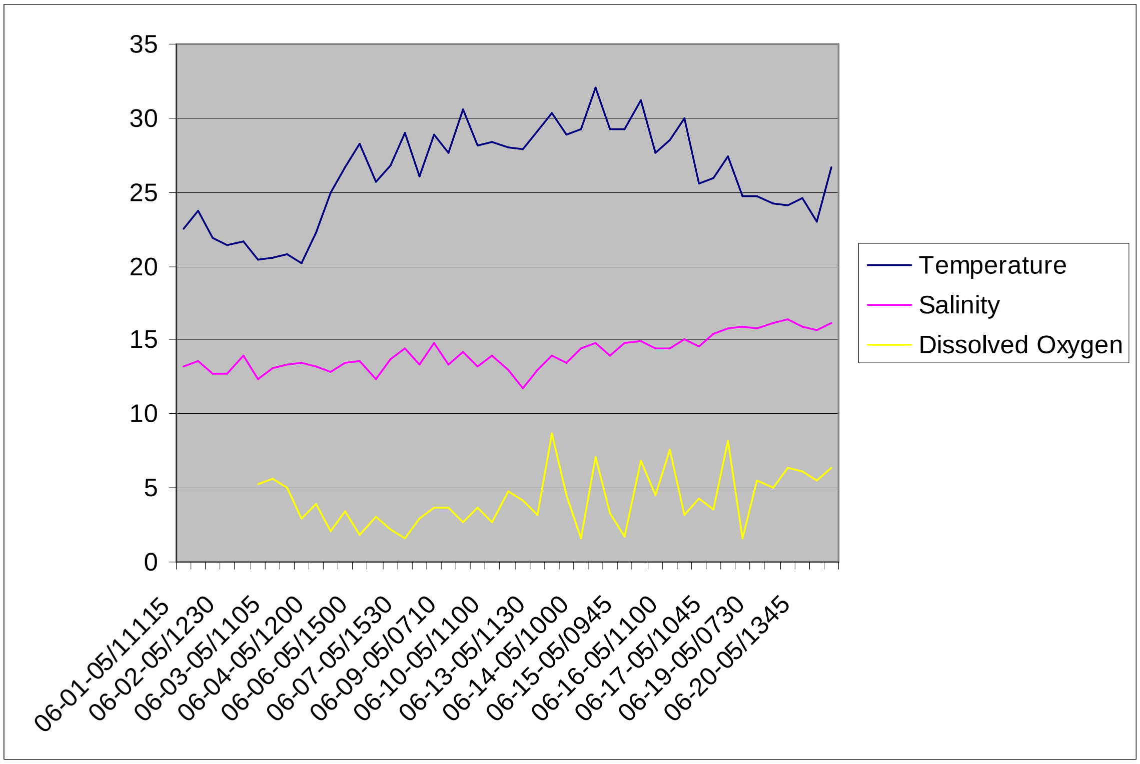


Figure 1. Water quality conditions from one representative basin (10,000) holding eelgrass reproductive material prior to and during seed release.

Table 1. Water quality conditions from one representative basin (10,000) holding eelgrass reproductive material prior to and during seed release.

Date/Time	Temperature (^{o}C)	Salinity (ppt)	Dissolved Oxygen (ppm)
06-01-05/11115	22.5	13.2	
06-01-05/1430	23.8	13.6	
06-02-05/0700	21.9	12.7	
06-02-05/1230	21.4	12.7	
06-02-05/1630	21.7	14	
06-03-05/0700	20.4	12.4	5.22
06-03-05/1105	20.6	13.1	5.6
06-03-05/1515	20.8	13.3	5.01
06-04-05/0715	20.2	13.5	2.9
06-04-05/1200	22.3	13.2	3.86
06-06-05/0715	25	12.9	2.1
06-06-05/1115	26.7	13.5	3.45

06-06-05/1500	28.3	13.6	1.8
06-07-05/0715	25.7	12.4	3.05
06-07-05/1130	26.8	13.7	2.23
06-07-05/1530	29	14.5	1.56
06-08-05/0730	26.1	13.4	2.99
06-08-05/1300	28.9	14.8	3.67
06-09-05/0710	27.6	13.4	3.72
06-09-05/1530	30.6	14.2	2.66
06-10-05/0730	28.2	13.2	3.7
06-10-05/1100	28.4	14	2.67
06-11-05/1000	28	13	4.81
06-13-05/0715	27.9	11.7	4.2
06-13-05/1130	29.1	13	3.21
06-13-05/1345	30.3	13.9	8.72
06-14-05/0715	28.9	13.5	4.5
06-14-05/1000	29.2	14.4	1.65
06-14-05/1445	32.1	14.8	7.12
06-15-05/0615	29.3	14	3.27
06-15-05/0945	29.3	14.8	1.73
06-15-05/1315	31.2	14.9	6.87
06-16-05/0715	27.7	14.4	4.57
06-16-05/1100	28.5	14.5	7.62
06-16-05/1430	30	15	3.23
06-17-05/0610	25.6	14.6	4.32
06-17-05/1045	25.9	15.4	3.61
06-17-05/1330	27.4	15.8	8.22
06-18-05/0700	24.7	15.9	1.53
06-19-05/0730	24.7	15.8	5.53
06-20-05/0615	24.2	16.1	5.02
06-20-05/1040	24.1	16.4	6.4
06-20-05/1345	24.6	15.9	6.17
06-21-05/0700	23	15.7	5.54
06-21-05/1400	26.7	16.2	6.42

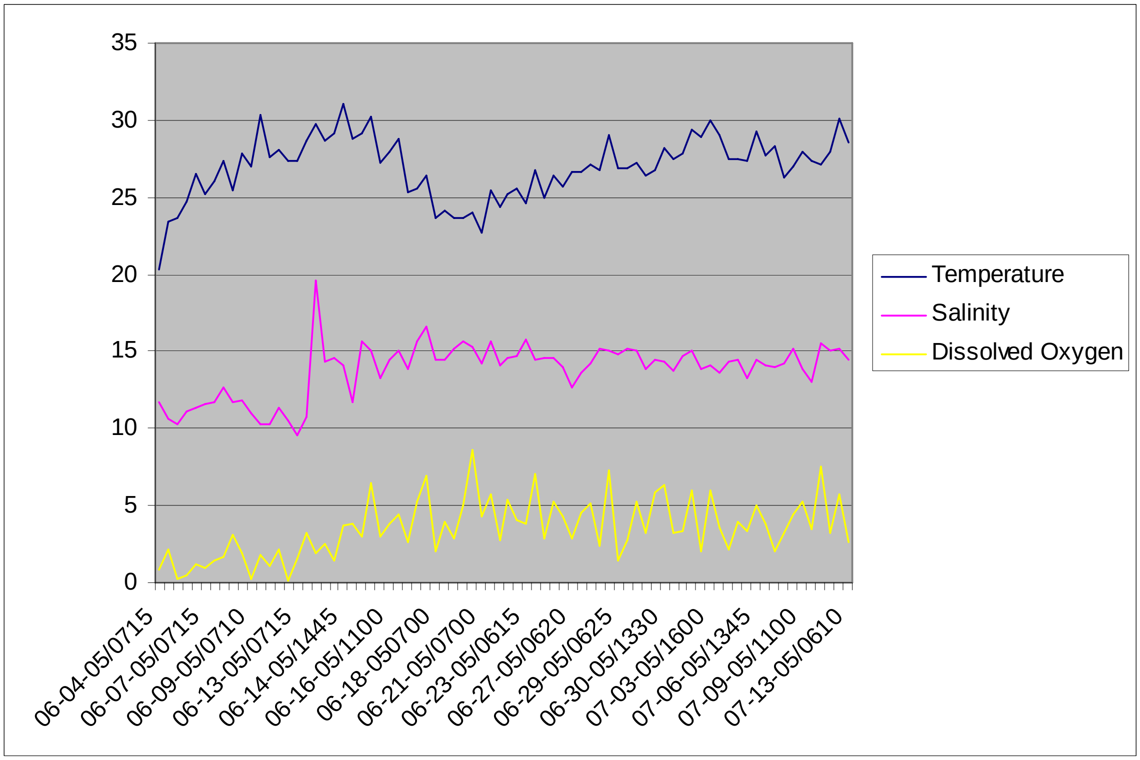


Figure 2. Water quality conditions of water from St. Georges Creek that is pumped into basins that are holding eelgrass seeds prior to broadcast.

Table 2. Water quality conditions from one representative basin (20,000) holding eelgrass reproductive material prior to and during seed release.

Date/Time	Temperature (°C)	Salinity (ppt)	Dissolved Oxygen (ppm)
06-04-05/0715	20.3	11.7	0.8
06-04-05/1200	23.4	10.6	2.14
06-06-05/0715	23.7	10.3	0.21
06-06-05/1115	24.7	11.1	0.52
06-06-05/1500	26.5	11.4	1.17
06-07-05/0715	25.2	11.6	0.91
06-07-05/1130	26.1	11.7	1.4
06-07-05/1530	27.4	12.7	1.65
06-08-05/0730	25.5	11.7	3.15
06-08-05/1300	27.8	11.8	1.97
06-09-05/0710	27	11	0.28
06-09-05/1530	30.4	10.3	1.75
06-10-05/0730	27.6	10.3	1.03
06-10-05/1100	28.1	11.4	2.14
06-11-05/1000	27.4	10.5	0.17
06-13-05/0715	27.3	9.6	1.52

06-13-05/1130	28.7	10.7	3.19
06-13-05/1345	29.8	19.6	1.93
06-14-05/0715	28.7	14.3	2.51
06-14-05/1000	29.2	14.6	1.4
06-14-05/1445	31.1	14.1	3.7
06-15-05/0615	28.8	11.7	3.81
06-15-05/0945	29.1	15.6	3
06-15-05/1315	30.2	15.1	6.5
06-16-05/0715	27.2	13.2	2.99
06-16-05/1100	28	14.5	3.83
06-16-05/1430	28.8	15.1	4.42
06-17-05/0610	25.3	13.8	2.62
06-17-05/1045	25.6	15.7	5.26
06-17-05/1330	26.4	16.6	6.87
06-18-050700	23.7	14.4	2
06-19-05/0730	24.1	14.4	3.9
06-20-05/0615	23.6	15.2	2.84
06-20-05/1030	23.7	15.6	5.06
06-20-05/1345	24	15.3	8.59
06-21-05/0700	22.7	14.2	4.31
06-21-05/1400	25.5	15.6	5.74
06-22-05/0610	24.4	14.1	2.72
06-22-05/1100	25.2	14.6	5.32
06-22-05/1340	25.6	14.7	4.08
06-23-05/0615	24.6	15.8	3.83
06-23-05/1330	26.7	14.5	7.02
06-24-05/0645	25	14.6	2.81
06-24-05/1150	26.4	14.6	5.29
06-25-05/0800	25.7	14	4.25
06-27-05/0620	26.6	12.7	2.86
06-27-05/1100	26.6	13.6	4.48
06-27-05/1345	27.1	14.2	5.13
06-28-05/0615	26.7	15.2	2.41
06-28-05/1336	29	15.1	7.23
06-29-05/0625	26.9	14.8	1.49
06-29-05/1000	26.9	15.2	2.73
06-29-05/1330	27.2	15.1	5.23
06-30-05/0615	26.4	13.9	3.17
06-30-05/1000	26.8	14.4	5.85
06-30-05/1330	28.2	14.3	6.37
07-01-05/0615	27.5	13.7	3.17
07-01-05/1000	27.8	14.7	3.3
07-01-05/1345	29.4	15	6.01
07-02-05/1100	28.9	13.9	2.01
07-03-05/1600	30	14.1	5.92
07-04-05/1230	29	13.6	3.62
07-05-05/0600	27.5	14.3	2.15
07-05-05/0945	27.5	14.5	3.92

07-06-05/0615	27.3	13.3	3.35
07-06-05/1345	29.3	14.5	5.04
07-07-05/0600	27.7	14.1	3.86
07-07-05/1330	28.3	14	1.99
07-08-05/0615	26.3	14.2	3.23
07-08-05/1345	27	15.2	4.41
07-09-05/1100	27.9	13.8	5.25
07-11-05/0610	27.3	13	3.45
07-11-05/1330	27.1	15.5	7.47
07-12-05/0600	28	15.1	3.25
07-12-05/1330	30.1	15.2	5.68
07-13-05/0610	28.5	14.5	2.59

Appendix B

St. George's Creek Water Quality Monitoring Results

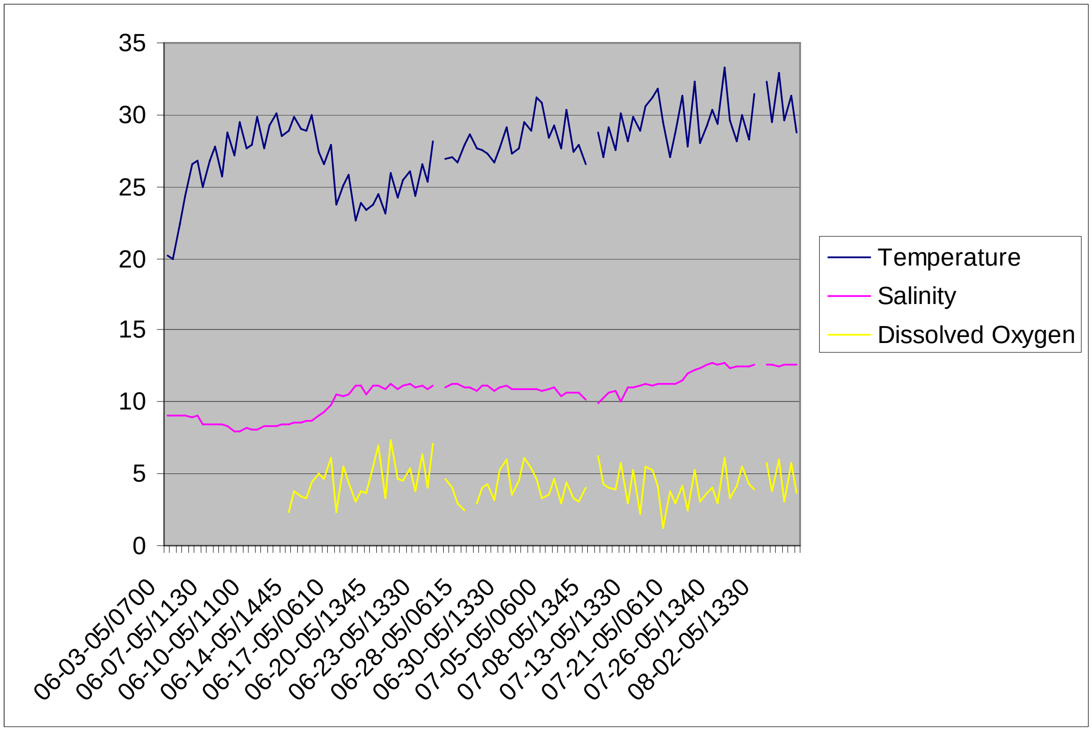


Figure 1. Water quality conditions of water from St. Georges Creek. Water from the creek is pumped into basins holding eelgrass seeds prior to broadcast.

Table 1. Water quality conditions of water from St. Georges Creek. Water from the creek is pumped into basins holding eelgrass seeds prior to broadcast.

Date	Time	Temperature (^{o}C)	Salinity (ppt)	Dissolved Oxygen (ppm)	Comments
10/3/2005	0800	21.4	15	5.8	
10/4/2005	1100	23.6	14.8	6.6	
10/5/2005	0730	23.1	15.1	5.7	
10/7/2005	0800	24.1	15.1	5.7	
10/11/2005	0800	20.9	14.3	5.2	
10/13/2005	0730	19.4	14.4	5.1	Rain
10/14/02005	0900	19.1	14.8	6.8	
10/17/2005	0830	15.9	14.9	6.9	
10/18/2005	1030	17.1	15.4	7.1	
10/19/2005	0730	16.9	15.4	5	
10/20/2005	0800	18.3	15.6	6.5	
10/21/2005	1300	17.6	14.8	7.3	Rain
10/27/2005	0700	13.5	15	7.5	
11/1/2005	0830	13.5	15.4	8.5	
11/2/2005	0830	13.1	15.1	8.6	
11/4/2005	0900	13.6	15.2	8	
11/8/2005	1000	15.8	14.5	6.8	

Literature Cited

Batiuk, R.A., Orth, R.J., Moore, K.A., Dennison, W.C., Stevenson, J.C., Staver, L.W., Carter, V., Rybicki, N.B., Hickman, R.E., Kollar, S., Bieber, S., Heasly, P. 1992. *Chesapeake Bay Submerged Aquatic Vegetation Habitat Requirements and Restoration Targets: A Technical Synthesis.* Chesapeake Bay Program, Annapolis, MD., CBP/TRS 83/92, Contract No. 68-WO-0043. 248 p.

Benoy, G.A., Kalff, J. 1999. Sediment accumulation and Pb burdens in submerged macrophytee beds. Limnology and Oceanography 44(4) 1081-1090.

Bigley, R.E. 1981. The population biology of two intertidal seagrasses, *Zostera marina* and *Ruppia maritima*, at Roberts Bank, British Columbia. Thesis, University of British Columbia, Vancouver British Columbia, Canada.

Borum, J. 1985. Development of epiphytic communities on eelgrass (*Zostera marina*) along a nutrient gradient in a Danish Estuary. Marine Biology 87:211-218.

Brush, G.S., Davis, F.W. 1984. Stratigraphic evidence of human disturbance in an estuary. Quaternary Research 22:91-108.

Brush, G.S., Hilgartner, W.B. 2000. Paleoecology of submerged macrophytes in the upper Chesapeake Bay. Ecological Monographs 70:645-667.

Burdick, D.M., Kendrick, G.A. 2001. Standards for seagrass collection, identification, and sample design. In: Short, F.T., Coles, R.G., editors. Global Seagrass Research Methods. Amsterdam: Elsevier Science BV. p 79-100.

Burt, J.S., Kendrock, G.A., Mansini, R. J., Simpson, C.J. 1995. Light and *Posidonia sinuosa* seagrass meadows in the temperate coastal waters of Western Australia: II. Effect of epiphyte species assemblage and biomass on attenuating light to the leaf surface. Technical Series 62. Department of Environmental Protection, Perth, Western Australia.

Cabin, R.J., Marshall, D.L., Mitchell, R.J. 2000. The demographic role of soil seed banks. II. Investigations of the fate of experimental seeds of the desert mustard *Lesquerella fendleri*. Journal of Ecology 88:293-302.

Cambridge, M.L., McComb, A.J. 1984. The loss of seagrasses in Cockburn sound, Western Australia. I. The time course and magnitude of seagrass decline in relation to industrial development. Aquatic Botany 20:229-243.

Chambers, J.C., MacMahon, J.A. 1994. A day in the life of a seed: movements and fates of seeds and their implications for natural and managed systems. Annual Review of Ecology and Systematics 25:263-292.

Cook, R.E. 1979. Patterns of juvenile mortality and recruitment in plants. In: Solibrig, O.T., Jain, S., Johnson, G.B., Raven, P.H., editors. Topics in Plant Population Biology. New York: Columbia University Press. p 207-231.

Den Hartog, C., Polderman, P.J.G. 1975. Changes in seagrass populations of the Dutch Waddenzee. Aquatic Botany 1:141-147.

Dennison, W.C., Albert, R.S. 1986. Photoadaptation and growth of *Zostera marina L*. (eelgrass) transplants along a depth gradient. Journal of Experimental Marine Biology and Ecology 98:265-282.

Dennison, W.C. 1987. Effects of light on seagrass photosynthesis, growth, and depth distribution. Aquatic Botany 27:15-26.

Dennison, W.C., Orth, R.J., Moore, K.A., Stevenson, J.C., Carter, V., Kollar, P.W., Bergstrom, P.W., Batiuk, R.A. 1993. Assessing water quality with submersed aquatic vegetation. BioSience 143:86-94.

Fishman, J.R., Orth, R.J. 1996. Effects of predation on *Zostera marina* seed abundance. Journal of Experimental Marine Biology Ecology 198:11-26.

Fonseca, M.S., Fisher, J.J., Zieman, J.C., Thayer. G.W. 1982. Influence of the seagrass, *Zostera marina* L., on current flow. Estuarine Coastal Shelf Science. 15:351-364.

Fonseca, M.S. 1992. Restoring seagrass in the Inited States. In: Thayer, G.W., editor. Restoring the nation's marine environment, Maryland Sea Grant, College Park, MD. p 79-110.

Fonseca, M.S., Kenworthy, W.J., Courtney, F.X., Hall, M.O. 1994. Seagrass planting in the southeastern United States: Methods for accelerating habitat development. Restoration Ecology 2(3):198-212.

Fonseca, M.S., Kenworthy, W.J., Thayer, G. 1998. Guidelines for the conservation and restoration of seagrasses in the United States and adjacent waters. In: NOAA Coastal Ocean Office, Silver Spring, MD. 222 p.

Gacia, E., Granata, T.C., Duarte, C.M. 1999. An approach to measurement of particle flux and sediment retention within seagrass meadows. Aquatic Botany 65:255-268.

Granger, S., Traber, M., Nixon, S.W., Keyes, R. 2002. *A practical guide for the use of seeds in eelgrass (Zostera marina L.) restoration. Part I. Collection, processing, and storage.* Schwartz, M. (editor), Rhode Island Sea Grant, Narragansett, R.I. 20 p.

Harper, J.L., Williams, J.T., Sagar, G.R. 1965. The behavior of seeds in soil. 1. The heterogeneity of soil surfaces and its role in determining the establishment of plants from seed. Journal of Ecology 53:273-286.

Harrison, P.G. 1987. Variations in success of eelgrass transplants over a five-years' period. Environmental Conservation 17(2):157-163.

Harwell, M.C., Orth, R.J. 1999. Eelgrass (*Zostera marina* L.) seed protection for field experiments and implications for large scale restoration. Aquatic Botany 64:51-61.

Inglis, G.J., Waycott, M. 2001. Methods for assessing seagrass ecology and population genetics. In: Short, F.T., Coles, R.G., editors. Global Seagrass Research Methods. Amsterdam: Elsevier Science BV. p 123-140.

Janzen, D.H. 1971. Seed predation by animals. Annual Review of Ecology and Systematics 2:465-492.

Kemp W.M., Boynton, W.R., Stevenson, J.C., Twilley, R.R., Means, J.C. 1983. The decline of submerged vascular plants in upper Chesapeake Bay: Summary of results concerning possible causes. Marine Technology Society Journal 17:78-89.

Kemp, W. M., W. R. Boynton, A. J. Hermann. 1995. Simulation models of an estuarine macrophyte ecosystem. pp. 262-278, In: Patten, B., Jørgensen, S.E., editors. Complex ecology. Prentice Hall, Englewood Cliffs, NJ.

Luckenbach, M.L., Orth, R.J. 1999. Effects of a deposit-feeding invertebrate on the entrapment of *Zostera marina* L. seeds. Aquatic Botany 62:235-247.

Madden, C., Day, J. 1992. Induced turbulence in rotating bottles affects phytoplankton productivity measurements in turbid waters. Journal of Plankton Research 14(8):1171-1191.

Maryland Department of Natural Resources (MD-DNR). 2005. *Maryland Tributary Strategy Patuxent River Basin Summary Report for 1985-2003 Data*. 61 p. [Electronic version.] Retrieved July 27, 2005 from http://www.dnr.state.md.us/bay/tribstrat/basin_summary_pax_012505.pdf

McRoy, C.P. 1968. The distribution and biogeography of *Zostera marina* (eelgrass) in Alaska. Pacific Science 22:507-513.

Moore, K.A., Orth, R.J., Nowak, J.F. 1993. Environmental regulation of seed germination in *Zostera marina* L. (eelgrass) in Chesapeake Bay: Effects of light, oxygen, and sediment burial depth. Aquatic Botany 45:79-91.

Moore, K.A., Neckles, H.A., Orth R.J. 1996. *Zostera marina* L. (eelgrass) growth and survival along a gradient of nutrients and turbidity in the lower Chesapeake Bay. Marine Ecology Progress Series 142:247-259.

Moore, K.A., Wetzel, R.L., Orth, R.J. 1997. Seasonal pulses of turbidity and their relations to eelgrass (*Zostera marina* L.) survival in an estuary. Journal of Experimental Marine Biology and Ecology 215:115-134.

Naylor, M.D., Kazyak, P. 1995. Quantitative characterization of submerged aquatic vegetation species in tidal freshwater reaches of the Patuxent River drainage basin. Submitted to Maryland Department of the Environment. Maryland Department of Natural Resources, Annapolis, MD. 24 p.

Olesen, B., Sand-Jensen, K. 1994. Patch dynamics of eelgrass *Zostera marina.* Marine Ecology Progress Series 106:147-156.

Olesen, B. 1999. Reproduction in Danish eelgrass (*Zostera marina L.*). Marine Ecology Progress Series 106:147-156.

Orth, R.J. 1975. Destruction of eelgrass, *Zostera marina* by the cownose ray, *Rhinoptera bonasus,* in Chesapeake Bay. Chesapeake Science 16:205-208.

Orth, R.J., Moore, K.A. 1983. Chesapeake Bay: An unprecedented decline in submerged aquatic vegetation. Science 222:51-53.

Orth, R.J., Luckenbach, M.W., Moore, K.A. 1994. Seed dispersal in a marine macrophyte: implications for colonization and restoration. Ecology 75:1927-1939.

Orth, R.J., Nowak, J.F., Wilcox, D.J., Whiting, J.R., Nagey, L.S. 1999. Distribution of submerged aquatic vegetation in the Chesapeake Bay and tributaries and Chincoteague Bay – 1998. Final Report, US Environmental Protection Agency, Annapolis, MD.

Orth, R.J., Harwell, M.C., Bailey, E.M., Batholomew, A., Jawad, J., Lombara, A., Moore, K., Rhode, J., Woods, H. 2000. A review of issues in seagrass seed dormancy and germination: Implications for conservation and restoration. Marine Ecology Progress Series 200:277-288.

Orth, R.J., Fishman, J.R., Harwell, M.C., Marion, S.R. 2003. Seed-density effects on germination and initial seedling establishment in eelgrass *Zostera marina* in the Chesapeake Bay region. Marine Ecology Progress Series 250:71-79.

Orth, R.J.**,** Bieri, J., Fishman, J.R., Harwell, M.C., Marion, S.R., Moore, K.A., Nowak, J.F., van Montfrans, J. In Press. A review of techniques using adult plants and seeds to transplant eelgrass (*Zostera marina* L.) in Chesapeake Bay and the Virginia Coastal Bays. Proc. Conf. Seagrass Restoration: Success, Failure, and the Costs of Both. March 11, 2003. Sarasota, Florida.

Parham, T., Karrh, L. 1998. *Maryland Submerged Aquatic Vegetation Restoration Targeting System.* Final Report to National Oceanic and Atmospheric Administration. Maryland Department of Natural Resources, Annapolis, MD. 32 p.

Patuxent River Commission Staff. 2003. Patuxent River Basin Summary. p 1-2.

Petticrew, E.L., Kalff, J. 1991. Predictions of surficial sediment composition in the littoral zone of lakes. Limnology and. Oceanography. 36:384-392.

Phillips, G.L., Emnison, D., Moss, B. 1978. A mechanism to account for macrophyte decline in progressively eutrophicated freshwaters. Aquatic Botany 4:103-126.

Pickerell, C., Schott, S., Wyllie-Echeverria, S. 2003. Buoy-deployed seeding: A new approach to restoring seagrass using seed. In: Submerged Aquatic Vegetation Propagation Workshop; 2003 September 3-4; Baltimore, MD. http://el.erdc.usace.army.mil/sav/workshop.html

Pickerell, C., Schott, S. and Wyllie-Echeverria, S. 2005. Buoy Deployed Seeding: Demonstration of a new eelgrass (*Zostera marina* L.) planting method. Ecological Engineering 25:127-136.

Rhoads, D.C., Young, D.K. 1970. The influence of deposit feeding organisms on sediment stability and community trophic structure. Journal of Marine Research 28:150-178.

Rhoads, D.C. 1974. Organism-sediment relations on the muddy sea floor. Oceanography and Marine Biology: An Annual Review. 12:263-300.

Silberhorn, G.M., Orth, R.J., Moore, K.A. 1983. Anthesis and seed production in *Zostera marina* L. (eelgrass) from the Chesapeake Bay. Aquatic Biology 15:133-144.

Stankelis, R.M., Boynton, W.R., Frank, J.M. 1999. Submerged aquatic vegetation (SAV) habitat evaluation. Boynton, W.R., Rohland, F.M., editors. Ecosystem Processes Component Level 1 Interpretive Report No 16. Chesapeake Biological Laboratory, University of Maryland System. p 77-117.

Stankelis, R.M., Naylor, M.D., Boynton, W.R. 2003. Submerged aquatic vegetation in the mesohaline region of the Patuxent estuary: Past, present, and future status. Estuaries 26:186-195.

Thom, R.M., Borde, A.B., Williams, G.D., Southared, J.A., Blanto, S.L., Woodruff, D.L. 2001. Effects of multiple stressors on eelgrass restoration projects. Proceedings of Puget Sound Research 2001, The fifth Puget Sound Research Conference, 2001.

Twilley, R.R., Kemp, W.M., Staver, K.W., Stevenson, J.C., Boynton, W.R. 1985. Nutrient enriched of estuarine submerged vascular plant communities. 1. Algal growth and effects on production of plants and associated communities. Marine Ecology Progress Series 23:179-191.

Wassenberg, T.J. 1990. Seasonal feeding on *Zostera capricorni* seeds by juvenile *Peneaus esculentus* (Crustacea: Decapoda) in Moreton Bay, Queensland. Australian Journal of Marine and Freshwater Research 41:301-310.

Wetzel, R.G., and Hough, R.A. 1973. Productivity and role of aquatic macrophytes in lakes: An assessment. Polish Archives of Hydrobiology 20:9-19.

Wetzel, R.G., Penhale, P.A. 1983. Production ecology of seagrass communities in the lower Chesapeake Bay. Marine Technology Society Journal 17:22-31.

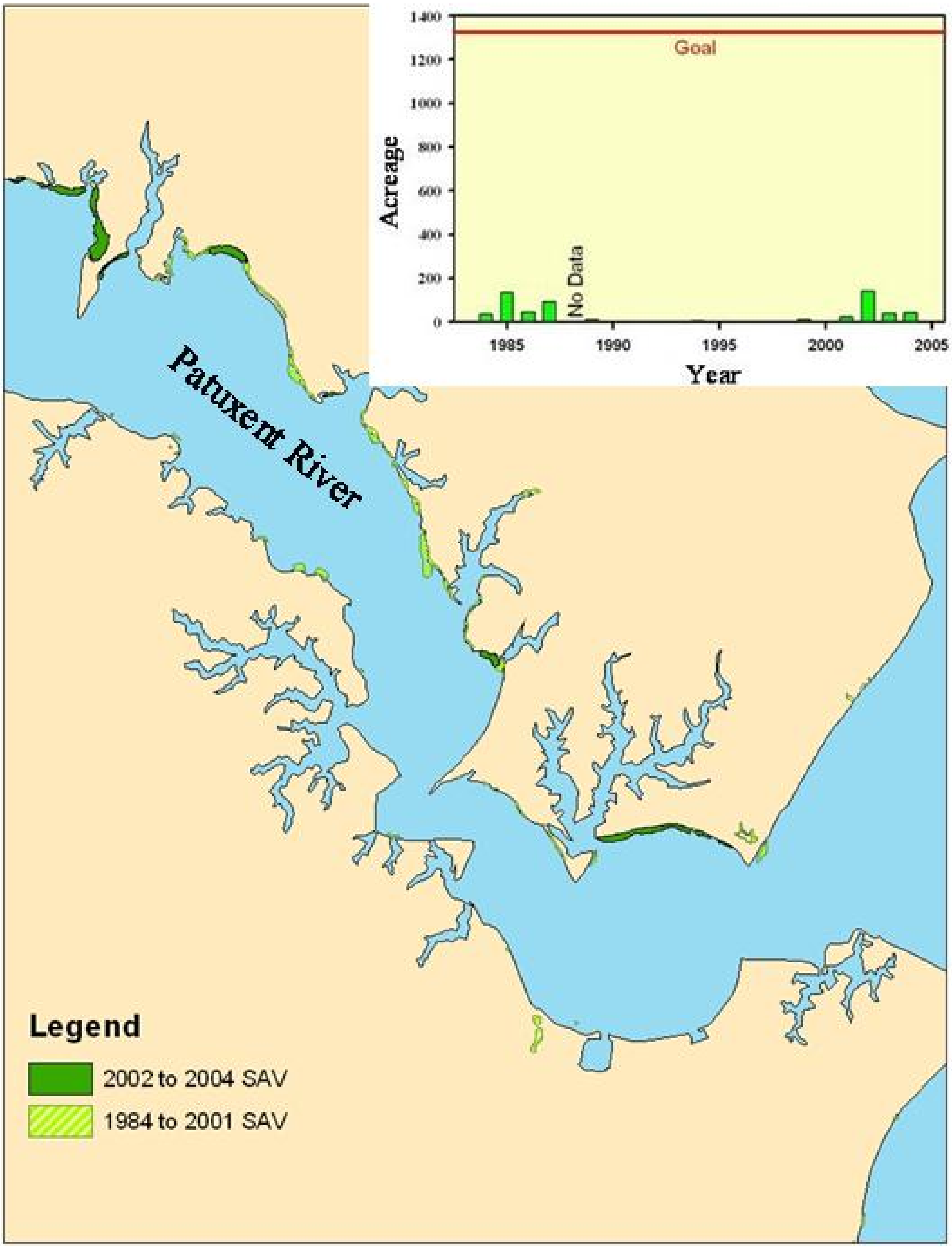


Figure 1. Map showing historic (1984-2001) and recent (2002-2004) bay grass coverage in the Patuxent River and a graph of the variation in total bay grass acreage in the mesohaline portion of the Patuxent River from 1984 to 2004.

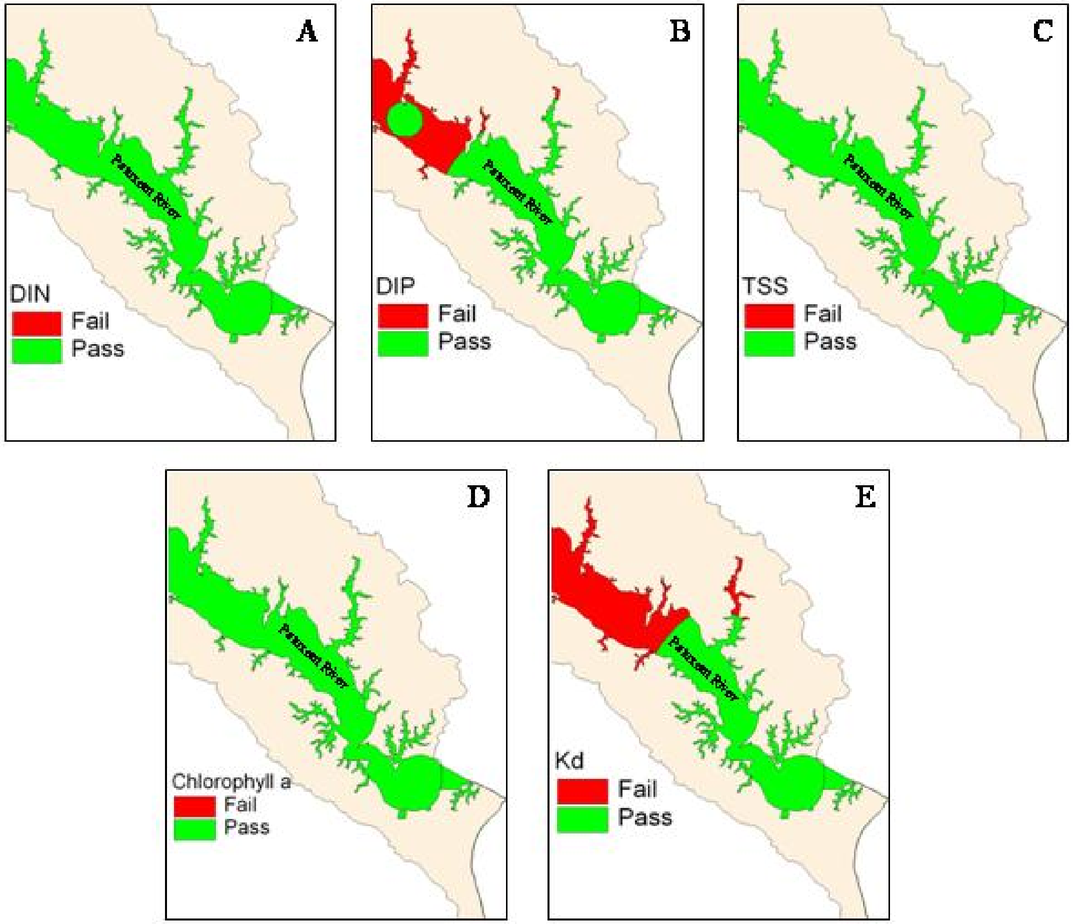


Figure 2. SAV habitat requirements in the Patuxent River. Each panel shows the status (pass/fail) of a particular requirement based on the requirements for SAV growth in the Chesapeake Bay and its tributaries (Batuik et al. 1992). A) Dissolved inorganic nitrogen (DIN), B) Dissolved inorganic phosphorus (DIP), C) Total suspended solids (TSS), D) Chlorophyll a, and E) Kd (light attenuation coefficient).

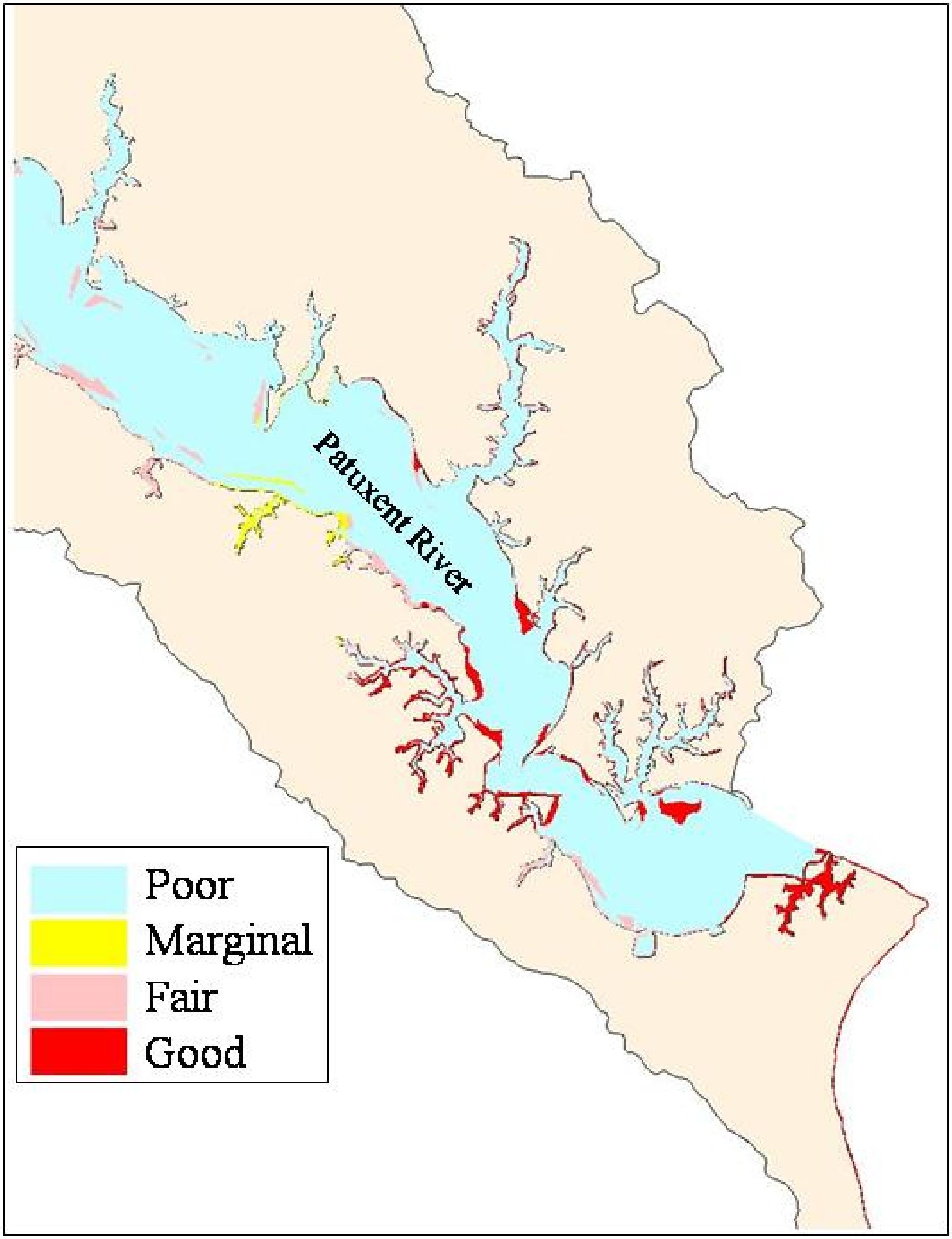


Figure 3. SAV restoration potential map of the Patuxent River, MD based on MD-DNR GIS based targeting model.

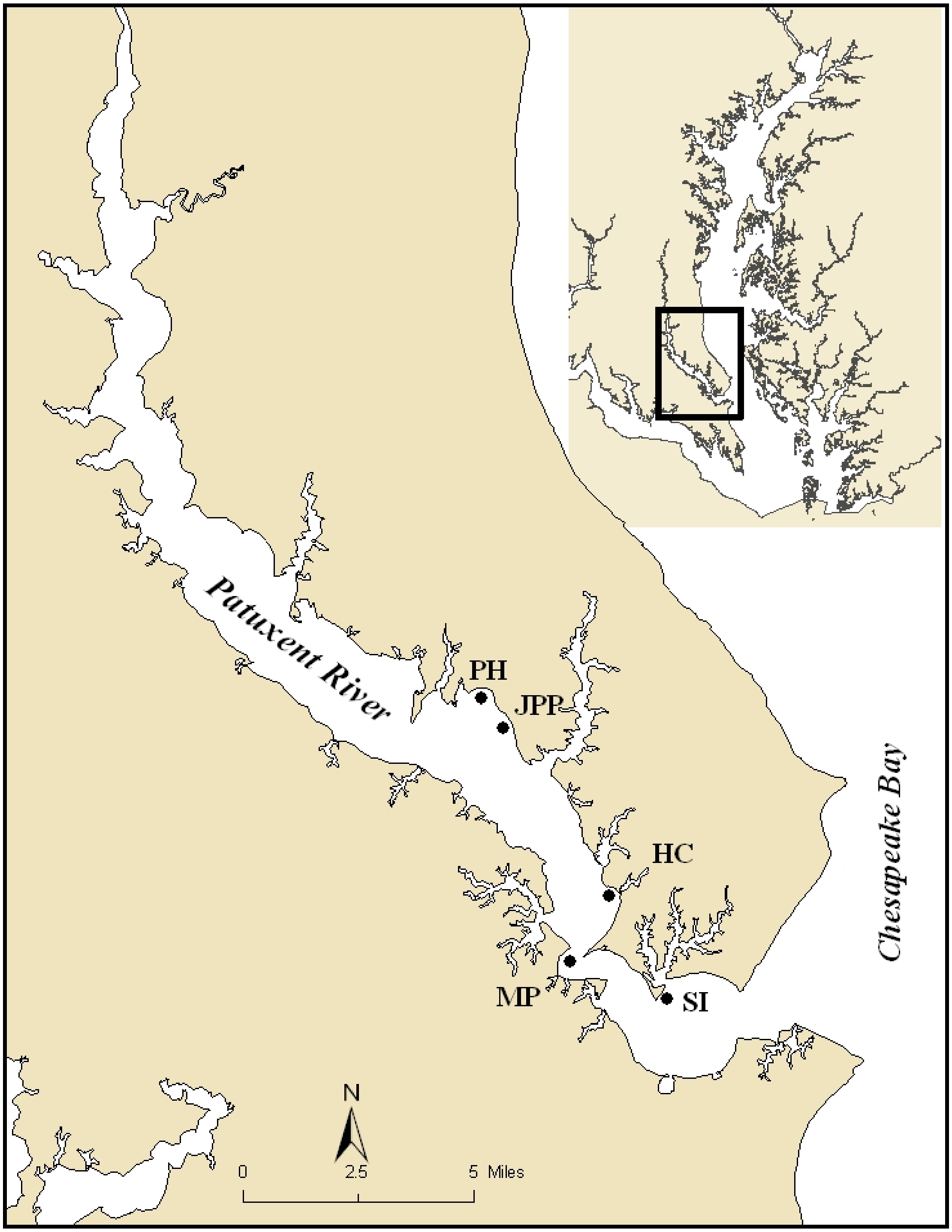


Figure 4. Map of Patuxent River, MD with restoration locations: Parrans Hollow (PH), Jefferson Patterson Park (JPP), Hungerford Creek (HC), Myrtle Point (MP), and Solomons Island (SI).

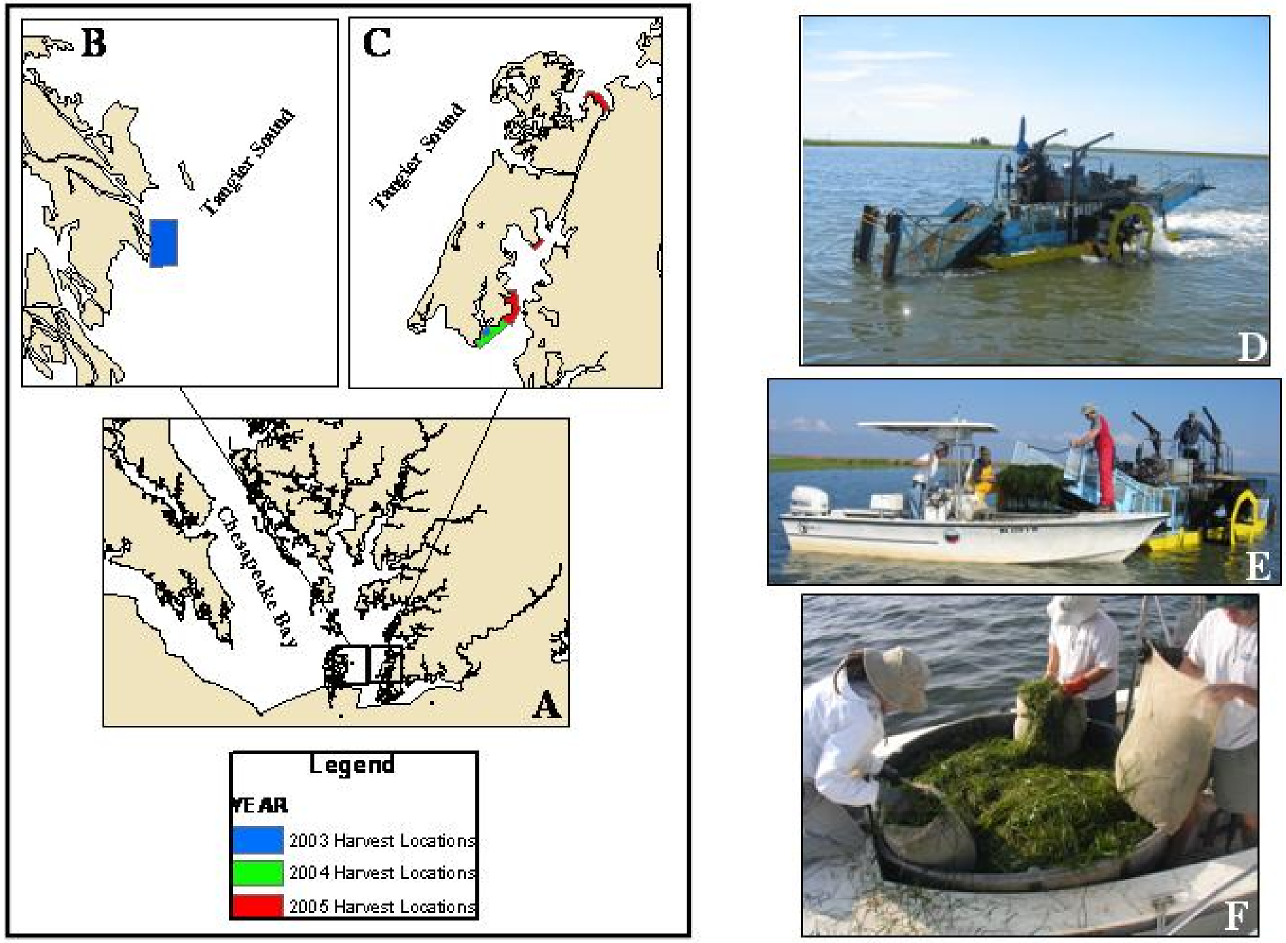


Figure 5. *Zostera marina* reproductive material collection 2003-2005. A) Map showing harvesting locations, B) Harvesting location near Smith Island in 2003, C) Harvesting locations near the Little Annemessex River in 2003 and 2004 and the Little Annemessex River and the mouth of Acre Creek in 2005, D) Mechanical harvest machine, E) MD DNR biologists unloading reproductive material from harvest boat, and F) MD DNR biologists and volunteers packing harvested material into mesh bags for transport.

Figure 6. A) Single spring seed bag with attached cinderblock, B) Double spring seed bag with attached cinderblock, C) Spring seed bag deployment, and D) Spring seed bag floating freely after deployment.

Figure 7. A) Manual fall seed broadcast method used in 2003, and B) Mechanical fall seed broadcast apparatus utilized during 2004 and 2005.

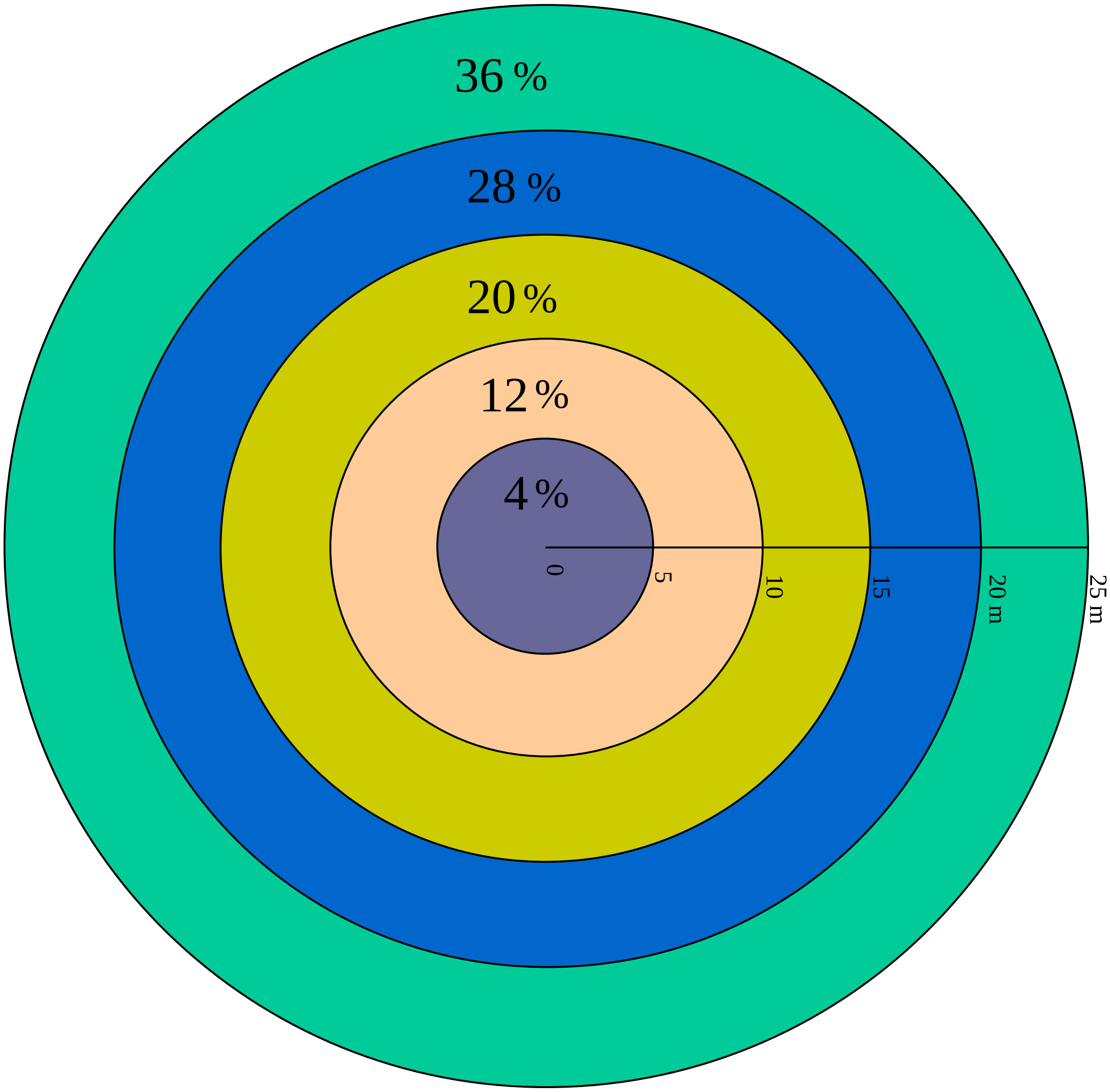


Figure 8. 25 meter radius (1/2 acre) plot divided into 5 meter concentric increments to ensure uniform distribution of seeds. The percent (%) of total area was used to determine the amount of seed material to be used in each 5 meter increment.

Figure 9. Patuxent River test plot success from May and July 2005 surveys. Adult *Z. marina* plants were transplanted into three - 1 m^2 test plots located adjacent to fall seed broadcast or spring seed bag areas at Parrans Hollow, Hungerford Creek, and Solomons Island on November 18, 2004. A density of 64 adult plants per m^2 was used for each test plot.

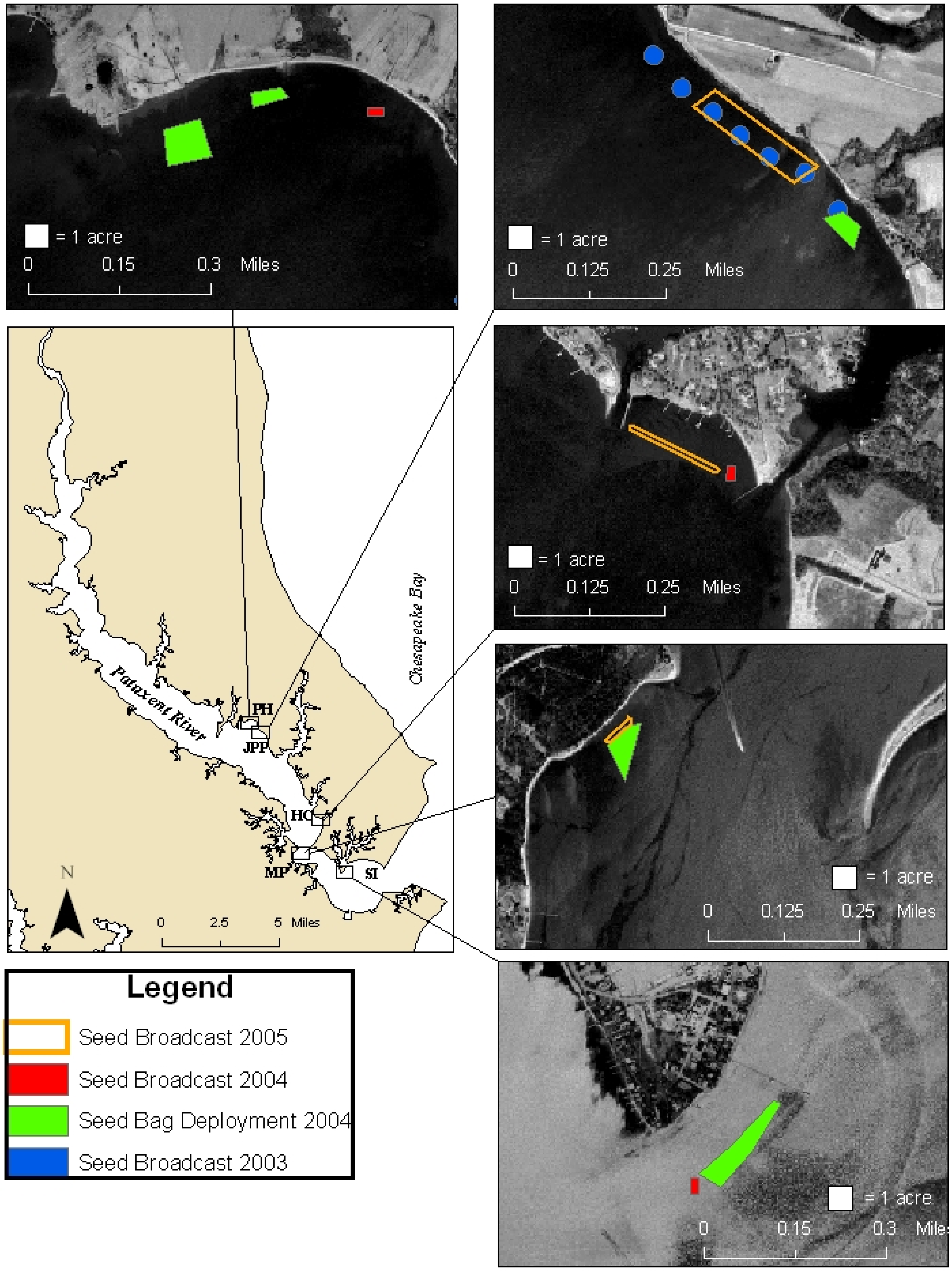


Figure 10. Map detailing seeding activity at each of five Patuxent River restoration locations: Parrans Hollow (PH), Jefferson Patterson Park (JPP), Hungerford Creek (HC), Myrtle Point (MP), and Solomons Island (SI) (2003-2005).

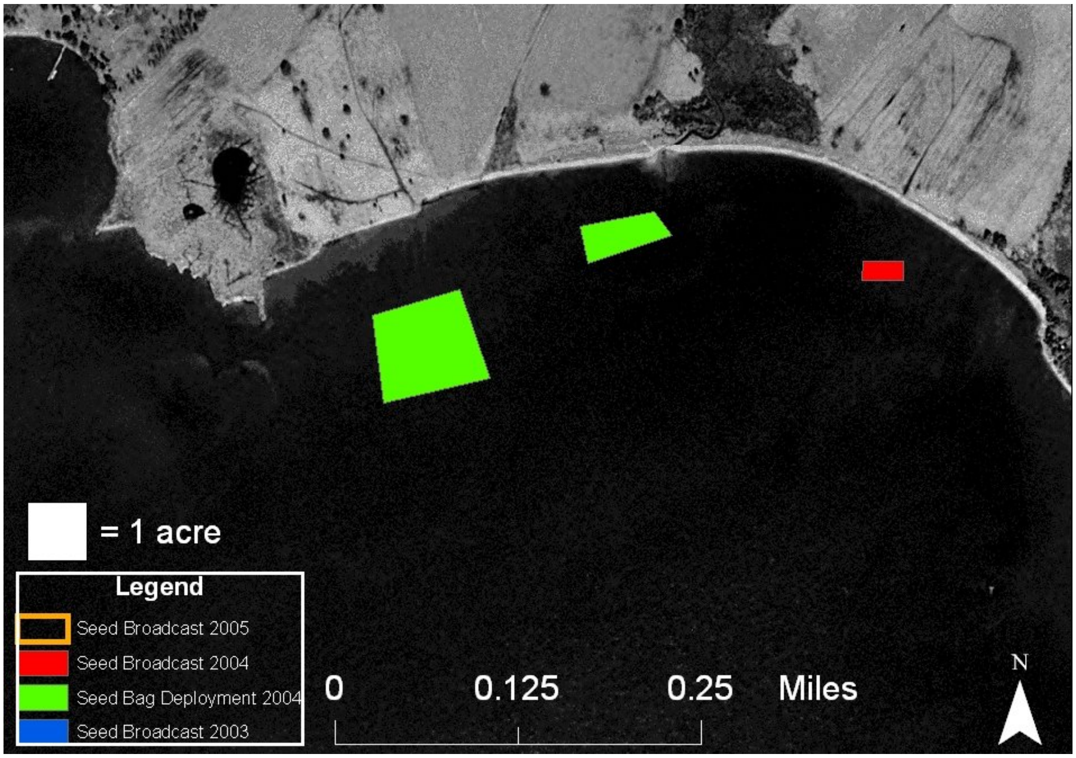


Figure 11. Map detailing seeding activity at the Parrans Hollow restoration location (2004).

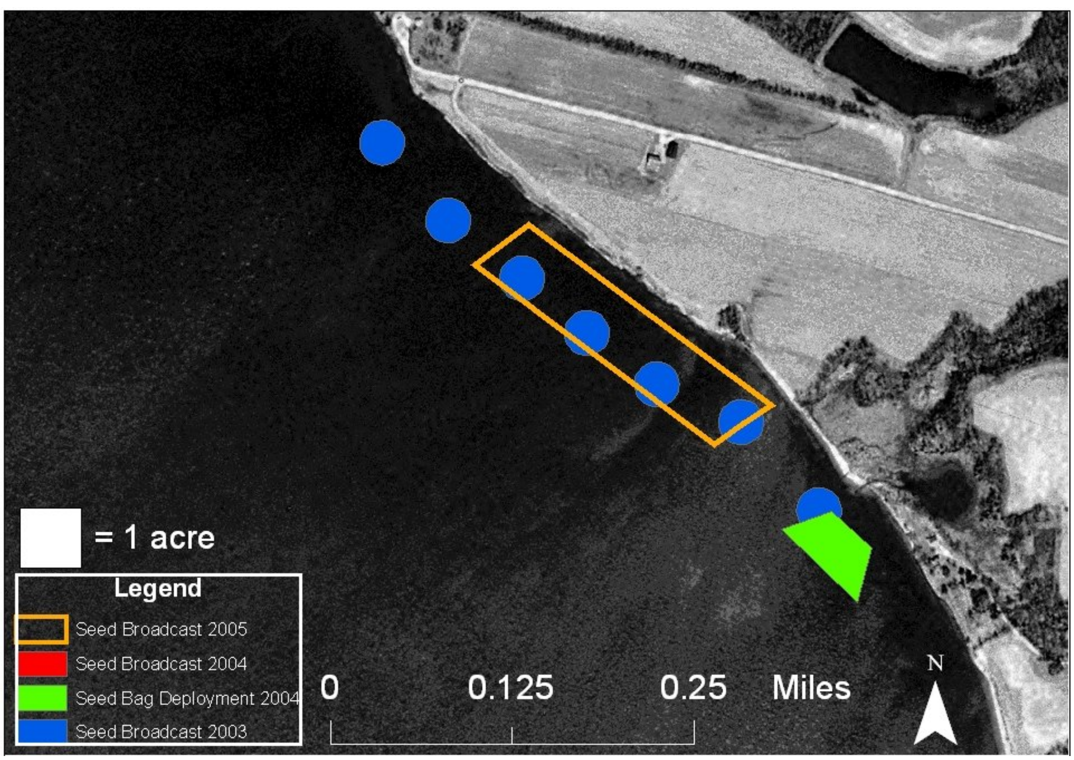


Figure 12. Map detailing seeding activity at the Jefferson Patterson Park restoration location (2003-2005).

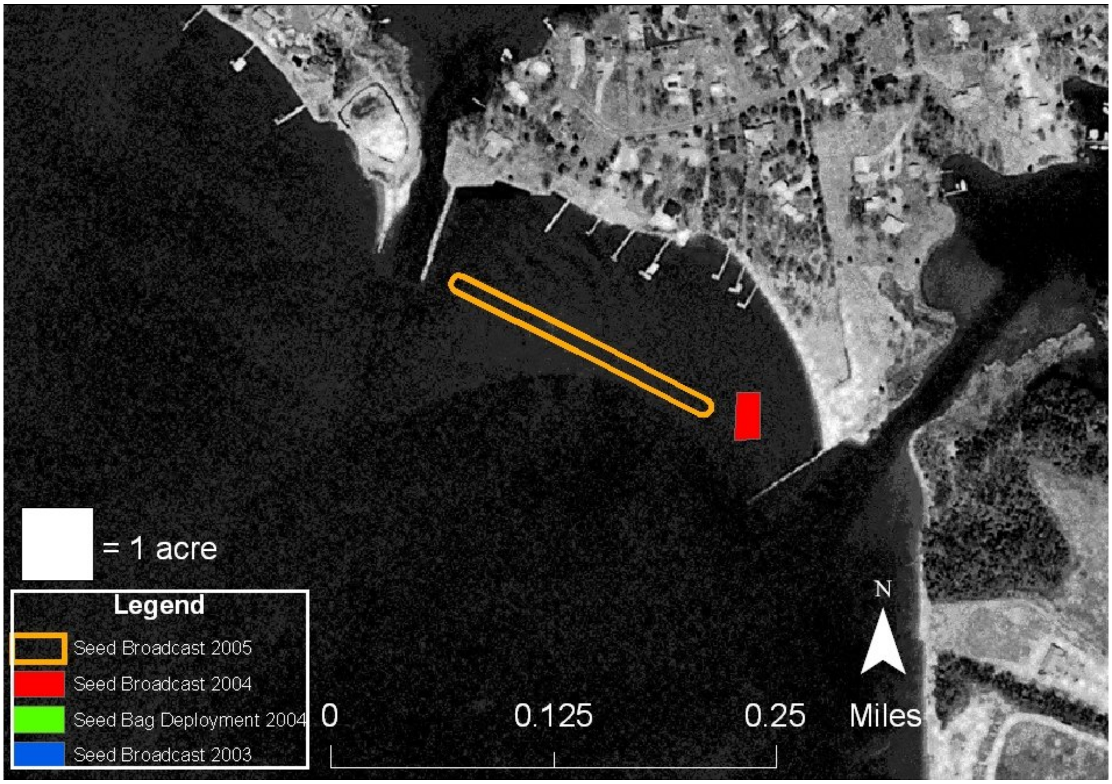


Figure 13. Map detailing seeding activity at the Hungerford Creek restoration location (2004-2005).

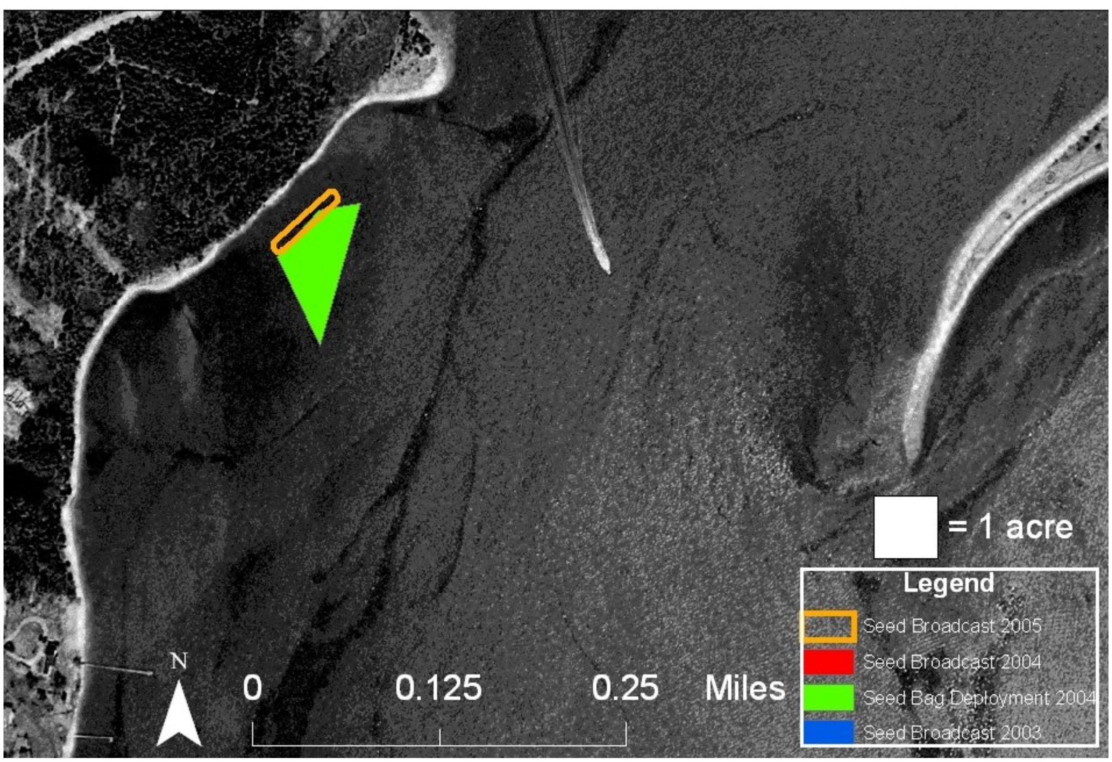


Figure 14. Map detailing seeding activity at the Myrtle Point restoration location (2004-2005).

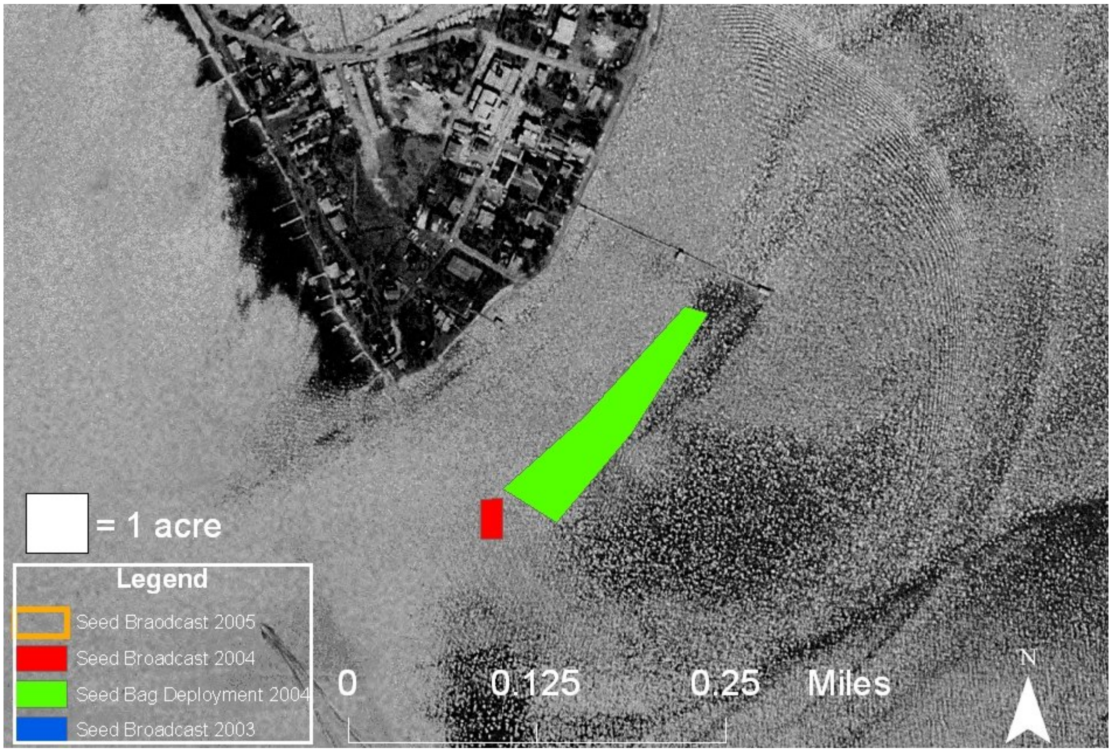
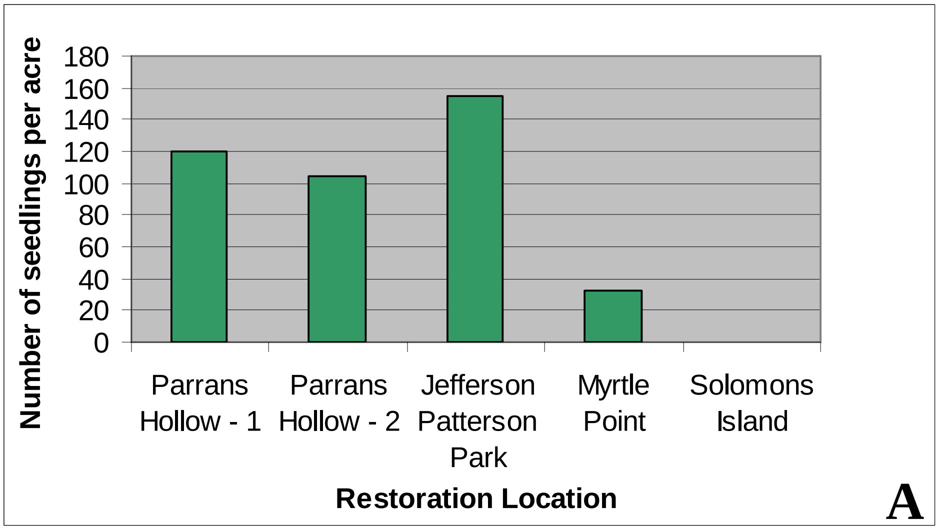


Figure 15. Map detailing seeding activity at the Solomons Island restoration location (2004).



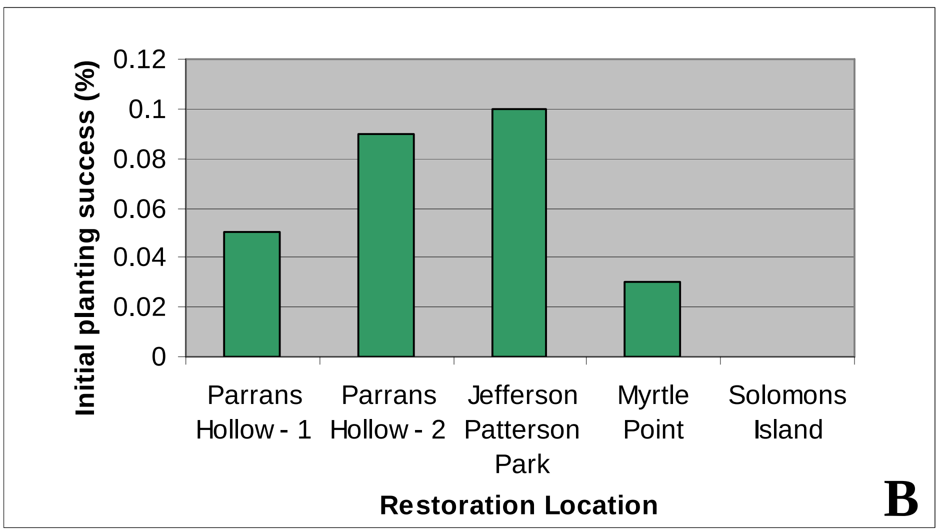


Figure 16. Success of the 2004 spring seed bag dispersal effort on the Patuxent River surveyed in May 2005. A) Number of seedlings observed per acre at each seed bag location (Number of seedlings observed = number of seedlings along the area of the survey transect * the total area of the seeded plot), and B) Initial planting success at each location (Initial planting success = total number of seedlings observed/the total number of seeds broadcast).

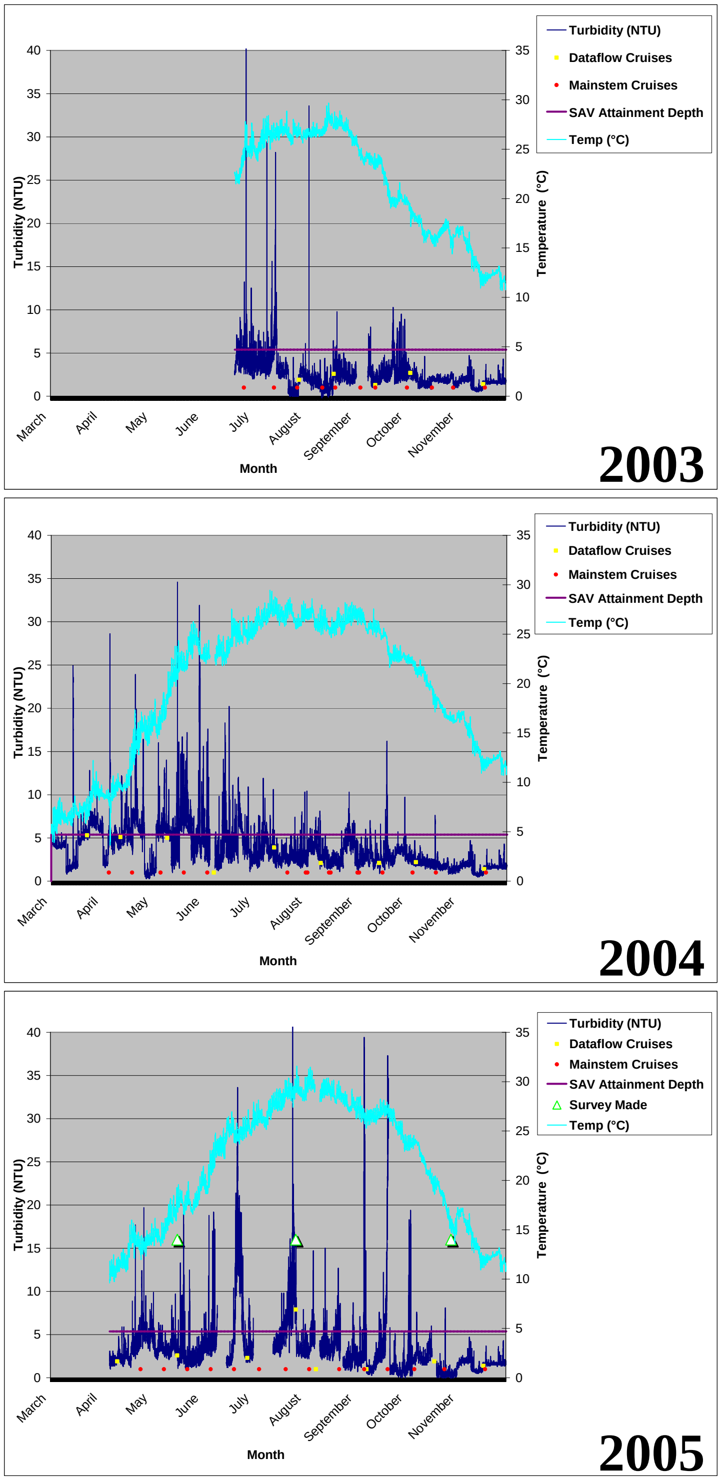


Figure 17. A continuous monitoring station was located at the Chesapeake Biological Laboratory dock near the Solomons Island restoration site on the Patuxent River to provide temporally intensive habitat assessments prior to and during restoration (2003-2005). Temperature and turbidity are presented for A) 2003, B) 2004, and C) 2005. The red line indicates a turbidity value of 5.38, the water clarity target for SAV that corresponds to 22% light penetration to a depth of 1m in the Patuxent River.

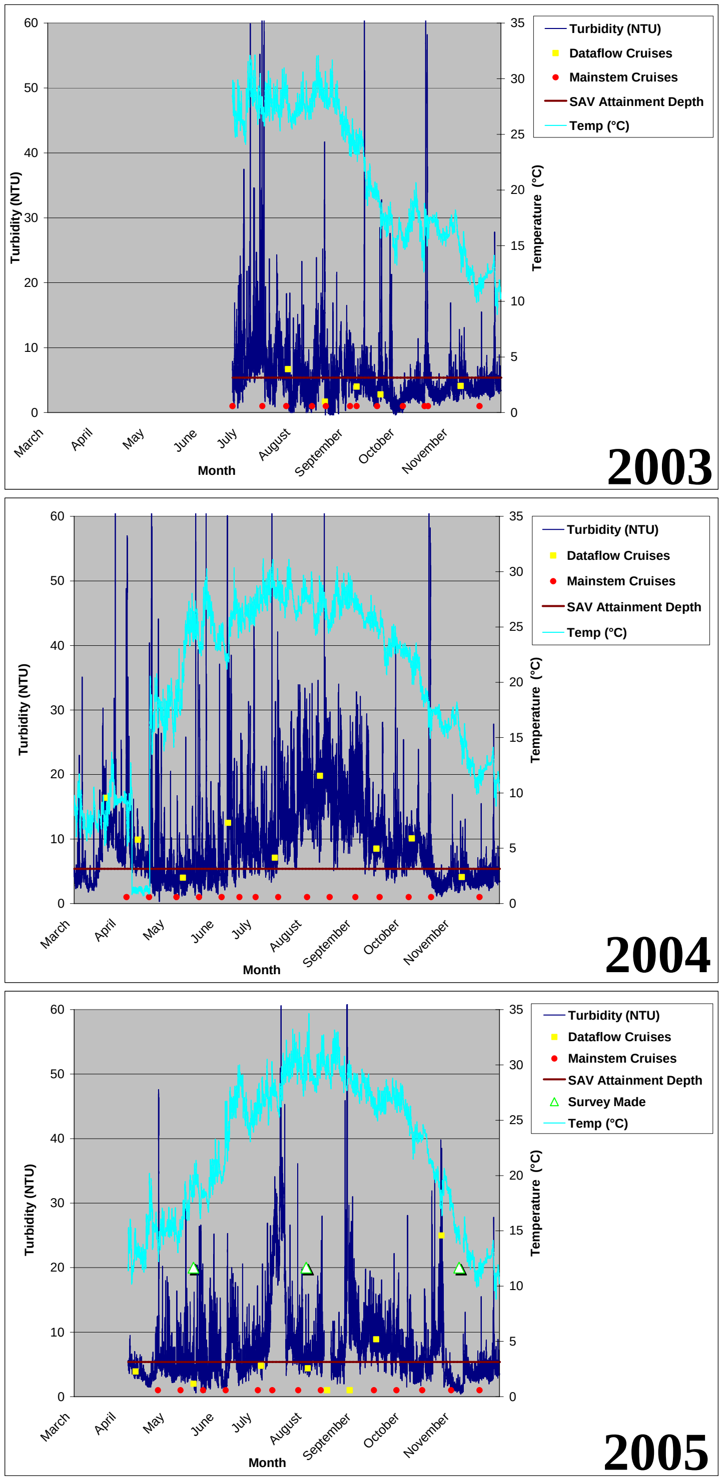


Figure 18. A continuous monitoring station was located at the Pin Oak Farm near the Jefferson Patterson Park and Parrans Hollow restoration sites on the Patuxent River to provide temporally intensive habitat assessments prior to and during restoration (2003-2005). Temperature and turbidity are presented for A) 2003, B) 2004, and C) 2005. The red line indicates a turbidity value of 5.38, the water clarity target for SAV that corresponds to 22% light penetration to a depth of 1m in the Patuxent River.

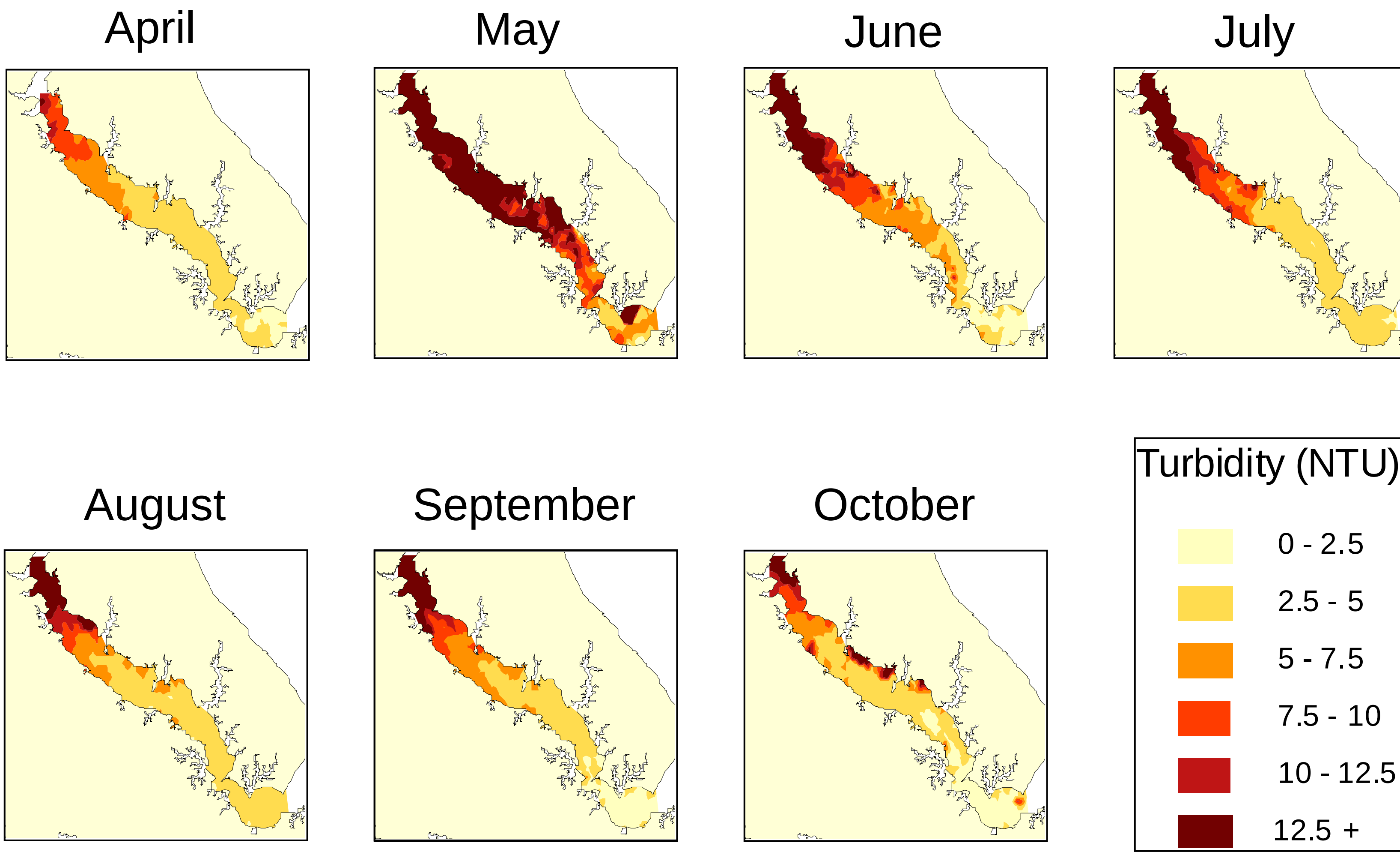


Figure 19. Turbidity data (NTU) from DATAFLOW cruises from April to October 2003 on the Patuxent River. DATAFLOW, a shipboard system of geospatial equipment and water quality probes, measures five water quality parameters from a flow-through stream of water collected near the water's surface.

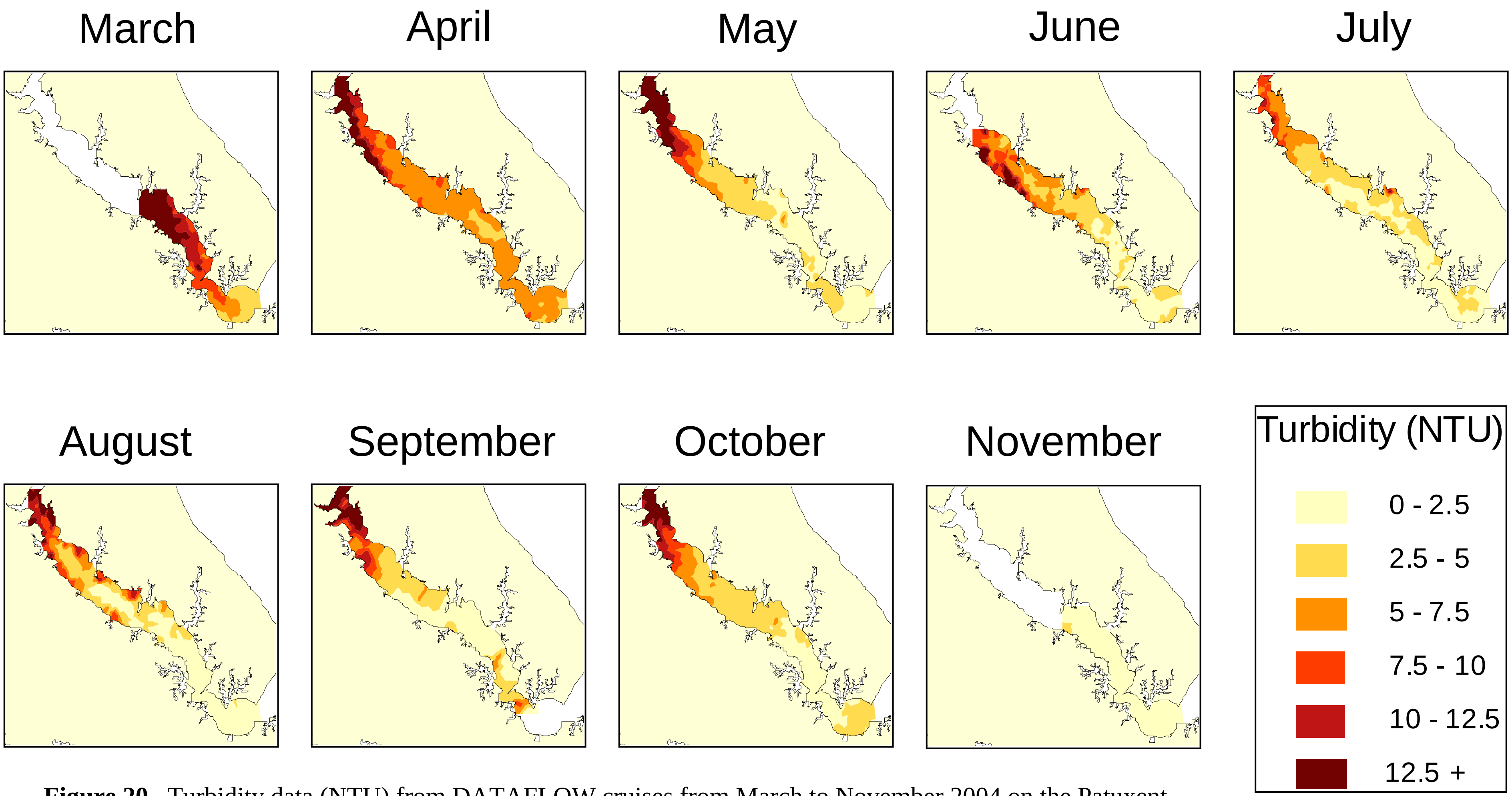


Figure 20. Turbidity data (NTU) from DATAFLOW cruises from March to November 2004 on the Patuxent River. DATAFLOW, a shipboard system of geospatial equipment and water quality probes, measures five water quality parameters from a flow-through stream of water collected near the water's surface.

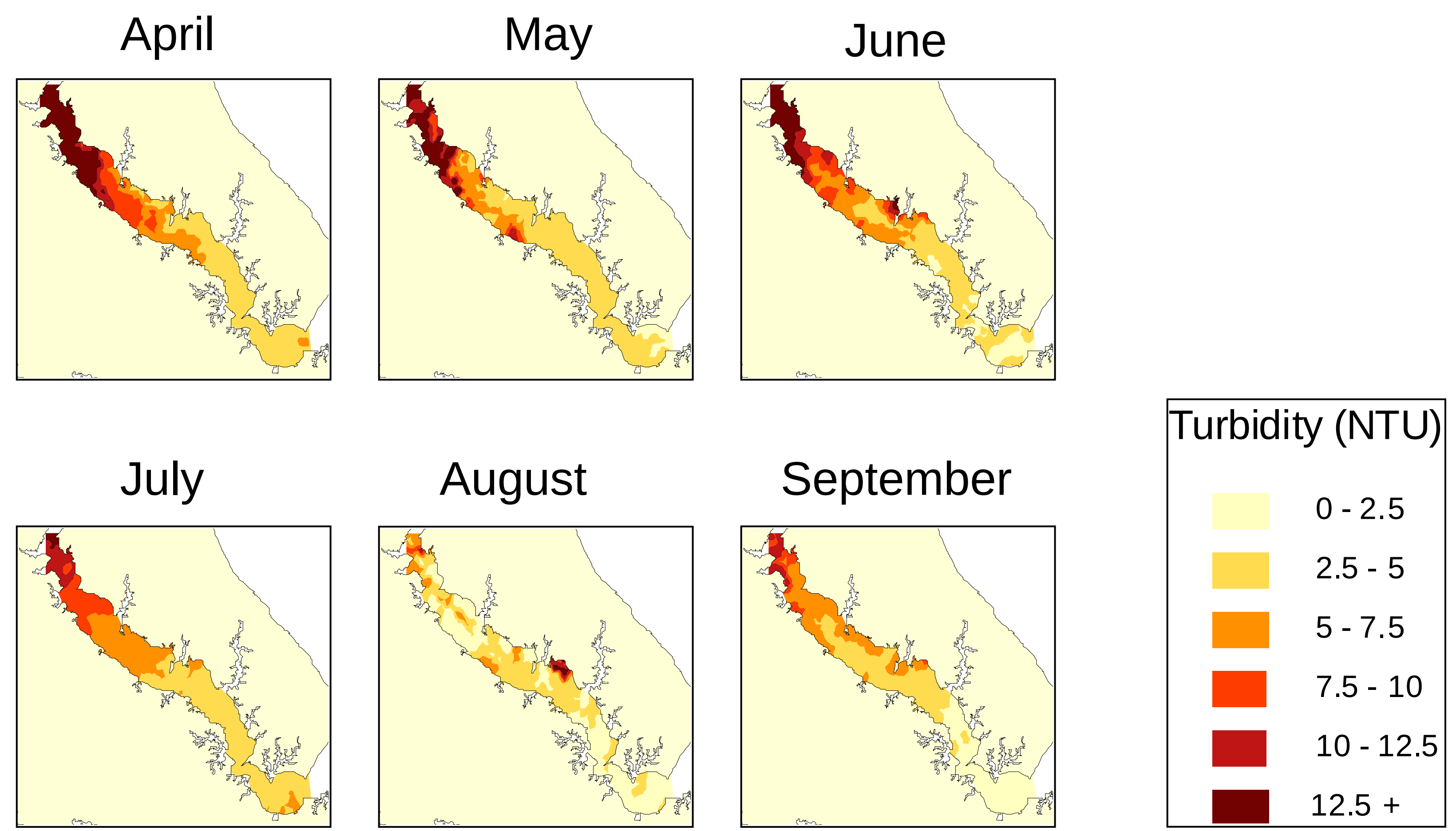


Figure 21. Turbidity data (NTU) from DATAFLOW cruises from April to September 2005 on the Patuxent River. DATAFLOW, a shipboard system of geospatial equipment and water quality probes, measures five water quality parameters from a flow-through stream of water collected near the water's surface.

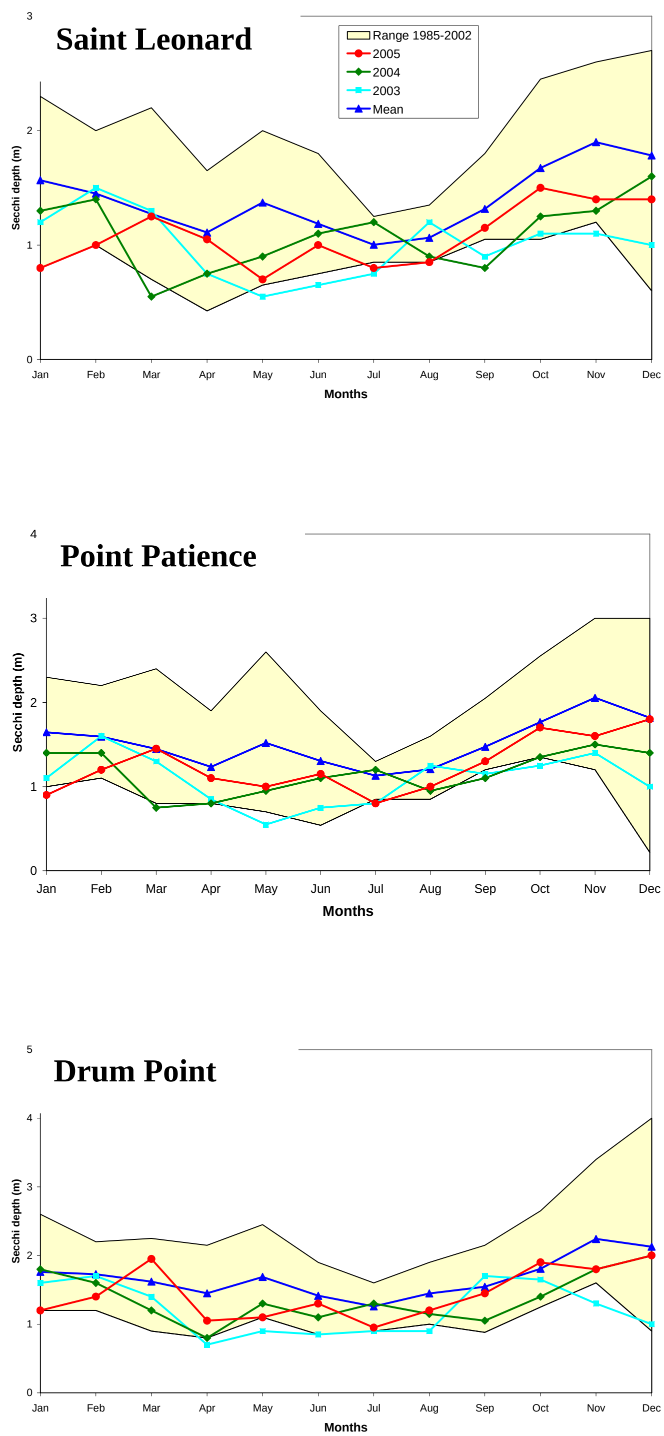


Figure 22. Secchi depth data for 2003, 2004, and 2005 compared to the range and mean of historical secchi depth values from 1985-2002 at three locations in the Patuxent River: Saint Leonard, Point Patience, and Drum Point.

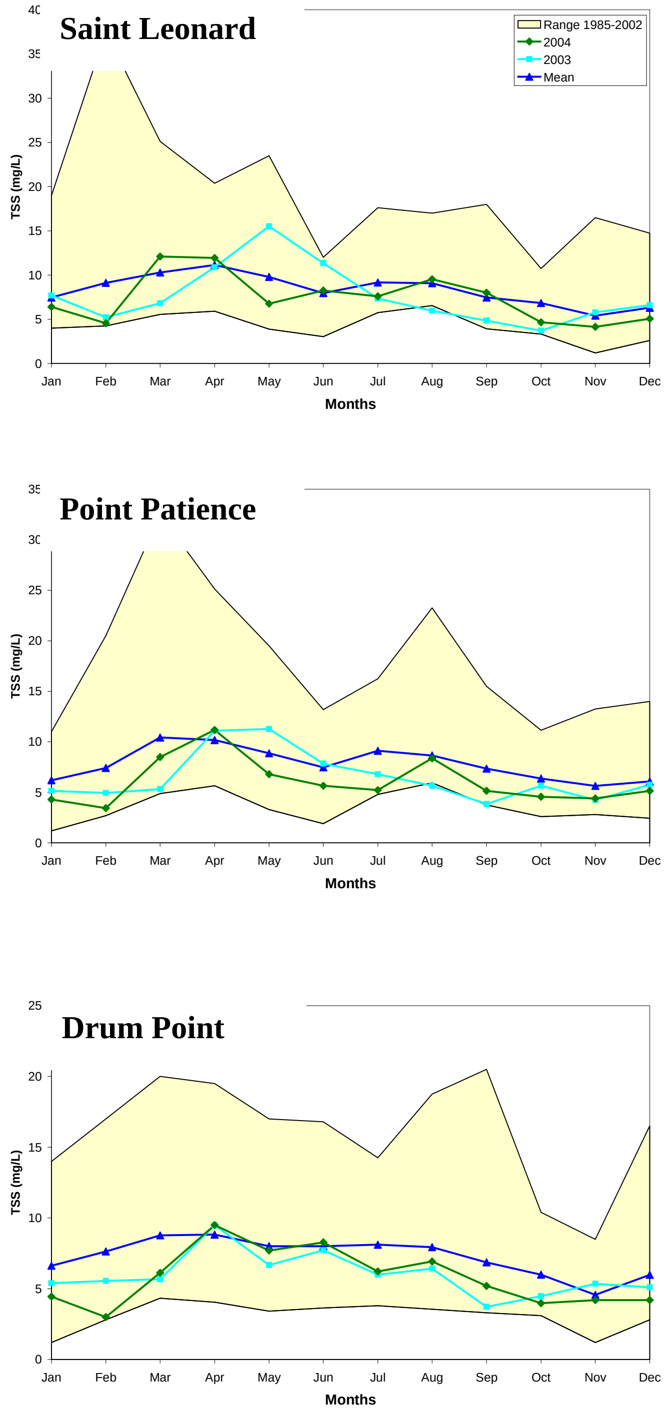


Figure 23. Total suspended sediments (TSS) data for 2003 and 2004 compared to the range and mean of historical TSS values from 1985-2002 at three locations in the Patuxent River: Saint Leonard, Point Patience, and Drum Point.

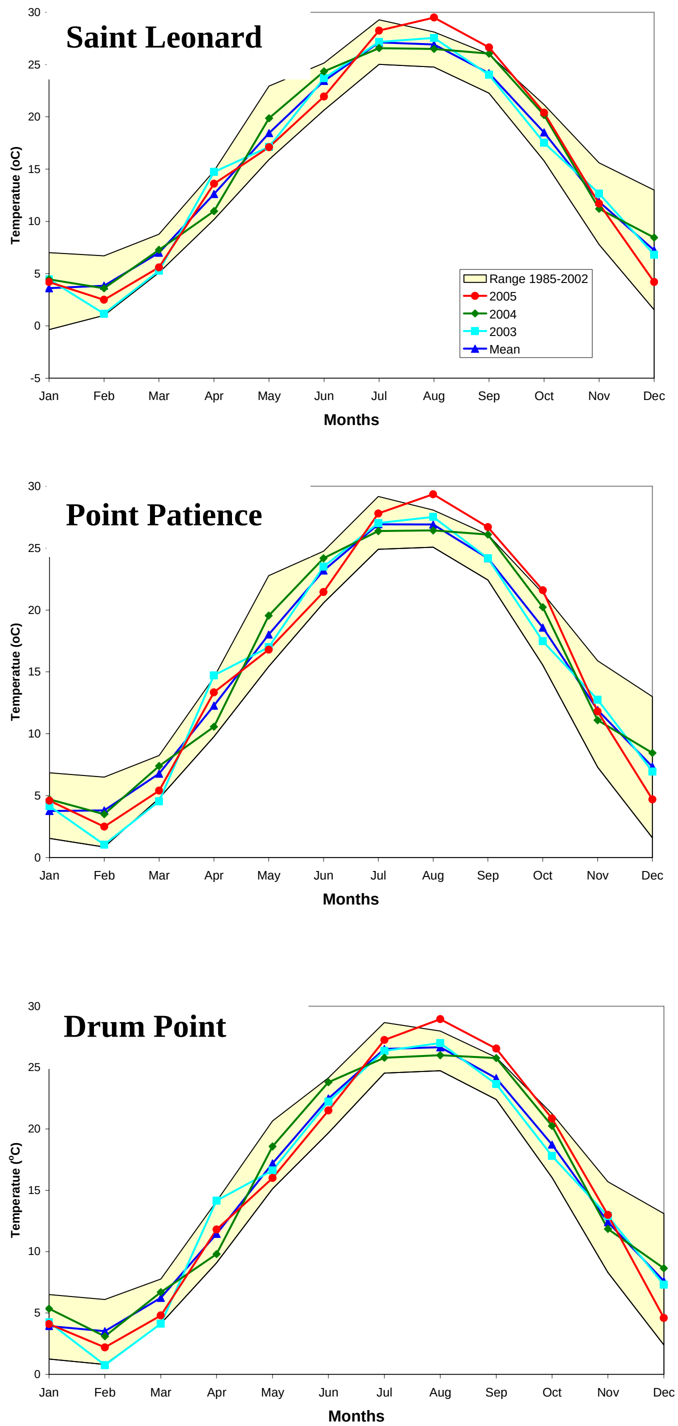


Figure 24. Temperature data for 2003 and 2004 compared to the range and mean of historical TSS values from 1985-2002 at three locations in the Patuxent River: Saint Leonard, Point Patience, and Drum Point.

Table 1. Details of eelgrass spring seed bag deployment in the Patuxent River (2004).

Site	Size (acres)	# of Seeds	Seeds/Acre
Parrans Hollow	5	605,000	121,000
Parrans Hollow	1	245,000	245,000
Jefferson Patterson Park	1	150,000	150,000
Myrtle Point	2.5	300,000	120,000
Solomons Island	5	605,000	212,000
	Size (acres)	**# Seeds**	
Total	14.5	1,905,000	

Table 2. Details of eelgrass fall seed broadcast in the Patuxent River (2003).

Site	**Size (acres)**	**# of Seeds**	**Seeds/Acre**
Jefferson Patterson Park	3	300,000	100,000
	Size (acres)	**# of Seeds**	
Total	3	300,000	

Table 3. Details of eelgrass fall seed broadcast in the Patuxent River (2004 and 2005).

Year	Site	Size (acres)	# of Seeds	Seeds/Acre
2004	Parrans Hollow	0.25	37,500	150,000
	Hungerford Creek	0.25	37,500	150,000
	Solomons Island	0.25	37,500	150,000
		Size(acres)	**# of Seeds**	
	Total	0.75	112,500	
2005	Jefferson Patterson Park	3	201,000	67,000
	Hungerford Creek	2	134,000	67,000
	Myrtle Point	0.5	33,500	67,000
		Size (acres)	**# of Seeds**	
	Total	5.5	368,500	

Table 4. Details of seed enumeration for the fall seed broadcast method (2003-2005). The total number of seeds harvested was calculated as the sum of the number of seeds per ml and the total volume of seeds collected. An estimate of the number of viable seeds was also determined.

Enumeration by Method	Year	Number of Total Seeds	Number of Viable Seeds Prior to Seed Storage (% of initial total seeds)	Number of Viable Seeds After Seed Storage (% of initial total seeds)
Fall Seed Broadcast	2003	2,300,000	No Data	250,000 (11%)
	2004	15,120,000	No Data	1,058,400 (7%)
	2005	12,373,500	7,446,000 (60%)	2,527,000 (20%)

Table 5. Results of 2004 test plot plantings (November 2004) on the Patuxent River, MD. The initial success rate was determined as the proportion of the original 64 plants that persisted in May 2005. The continued success rate in July 2005 was determined as the proportion of the plants that survived from the May 2005 survey.

		Parrans Hollow	Hungerford Creek	Solomons Island
Plants Observed May	Test Plot A	43	25	52
	Test Plot B	54	30	54
	Test Plot C	51	30	51
	Average Plants Observed	**49**	**28**	**52**
Initial Success Rate May	Test Plot A	67	39	81
	Test Plot B	84	47	84
	Test Plot C	80	47	80
	Average Success Rate (%)	**77**	**44**	**82**
Plants Observed July	Test Plot A	15	3	4
	Test Plot B	5	4	3
	Test Plot C	0	15	0
	Average Plants Observed	**7**	**7**	**2**
Continued Success Rate July	Test Plot A	35	12	8
	Test Plot B	9	13	6
	Test Plot C	0	50	0
	Average Success Rate (%)	**15**	**25**	**4**

Table 6. Compilation of all eelgrass restoration efforts in the Patuxent River by restoration site (2003-2005).

Restoration Site	Year	Broadcast Method	Size (Acres)	Number of Seeds	Seeds/Acre
Parrans Hollow	2004	Fall Seed Broadcast	0.25	37,500	150,000
		Spring Seed Bag	5	605,000	121,000
			1	245,000	245,000
			Total Acres	**Total Number of Seeds**	
			6.25	887,500	
Jefferson Patterson Park	2005	Fall Seed Broadcast	3	201,000	67,000
	2004	Spring Seed Bag	1	150,000	150,000
	2003	Fall Seed Broadcast	3	300,000	100,000
			Total Acres	**Total Number of Seeds**	
			7	501,000	
Hungerford Creek	2005	Fall Seed Broadcast	2	134,000	67,000
	2004	Fall Seed Broadcast	0.25	37,500	150,000
			Total Acres	**Total Number of Seeds**	
			2.25	171,500	
Myrtle Point	2005	Fall Seed Broadcast	0.5	33,500	67,000
	2004	Spring Seed Bag	2.5	300,000	120,000
			Total Acres	**Total Number of Seeds**	
			3	333,500	
Solomons Island	2004	Fall Seed Broadcast	0.25	37,500	150,000
		Spring Seed Bag	5	605,000	212,000
			Total Acres	**Total Number of Seeds**	
			5.25	642,500	

Table 7. Summary of spring seed bag dispersal (June 2 and 4, 2004) results in the Patuxent River, MD. Using SCUBA, eelgrass seedlings were enumerated the following spring (May 2005) along two or three diagonal, non-destructive, 1m^2, belt transects across the study plots. The total number of seedlings along the 1m^2 transects was then used to extrapolate the number of seedlings present throughout the total area (m^2) of the seeded plot. Initial planting success was then determined as the proportion of the total seedlings observed to the total seeds dispersed in the plot.

Restoration Location	Area Planted (m^2)	Total Seeds Dispersed	Seedlings Observed (May)	Area Surveyed (m^2)	Average Seedlings/m^2	Seedlings in plot	Initial Planting Success (%)
Parrans Hollow-1	20,234	605,000	7	296	0.03	519	0.09
			7	301			
			9	301			
		TOTAL	**23**	**897**			
Parrans Hollow-2	4,047	245,000	2	205	0.03	120	0.05
			8	184			
			7	184			
		TOTAL	**17**	**573**			
Jefferson Patterson Park	4,047	150,000	6	106	0.04	155	0.10
			2	103			
		TOTAL	**8**	**209**			
Myrtle Point	10,118	300,000	0	146	0.01	80	0.03
			2	107			
		TOTAL	**2**	**253**			
Solomons Island	20,235	605,000	0	48	0.00	0	0.00
			0	49			
		TOTAL	**0**	**97**			

Table 8. Turbidity and Temperature data from two continuous monitoring stations in the Patuxent River are displayed graphically in Figures 17 (CBL) and 18 (Pin Oak). The red line on those graphs indicates an NTU of 5.38, the water clarity target for SAV that corresponds to 22% light penetrating to a depth of 1m in the Patuxent River. The percentage of time that turbidity exceeded this 5.38 NTU is presented for both the entire data set as well as the eelgrass growing season (March 1-October 31, where the full data set available). The percentage of time that temperature exceeded 30^{o}C and 25^{o}C, two upper temperature threshold limits for eelgrass plants to thrive, is presented for both the entire data set as well as the eelgrass growing season (March 1-October 31, where the full data set available). Both turbidity and temperature limits were also examined between the May 12th and July 26th survey dates in 2005.

				% Exceeding limit	
			Dates	**CBL Station**	**Pin Oak Station**
Turbidity	2003	Overall	June 20 - Nov 10	4.5	41.9
		Growing Season	June 20-Oct 31	4.6	45.6
	2004	Overall	March 1 - Nov 29	18.0	64.6
		Growing Season	March 1 -Oct 31	20.1	70.9
	2005	Overall and Growing Season	Apr 6 - Oct 31	13.2	54.7
		Between Surveys	May 12- July 26	24.8	62.7
Temperature (30 ^{o}C)	2003	Overall	June 20 - Nov 10	0.0	3.7
		Growing Season	June 20-Oct 31	0.0	4.0
	2004	Overall	March 1 - Nov 29	0.0	0.5
		Growing Season	March 1 -Oct 31	0.0	0.6
	2005	Overall and Growing Season	Apr 6 - Oct 31	1.6	8.0
		Between Surveys	May 12- July 26	0.3	9.1
Temperature (25 ^{o}C)	2003	Overall	June 20 - Nov 10	39.5	60.1
		Growing Season	June 20-Oct 31	39.5	65.7
	2004	Overall	March 1 - Nov 29	31.1	38.2
		Growing Season	March 1 -Oct 31	31.1	42.8
	2005	Overall and Growing Season	Apr 6 - Oct 31	45.9	52.3
		Between Surveys	May 12- July 26	46.6	61.3